48 part 1

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 March 2002 (28.03.2002)

PCT

(10) International Publication Number
WO 02/24747 A2

(51) International Patent Classification[7]: C07K 14/575, C12N 9/64, C07K 14/72, C12N 15/10, 5/10, C07K 16/18, A01K 67/027, C12Q 1/68, G01N 33/53, A61K 39/00, 39/395, 48/00

(21) International Application Number: PCT/EP01/10087

(22) International Filing Date: 31 August 2001 (31.08.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
00120123.5 19 September 2000 (19.09.2000) EP

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: POLYMORPHISMS IN HUMAN GENES OF CARDIOVASCULAR REGULATORS AND THEIR USE IN DIAGNOSTIC AND THERAPEUTIC APPLICATIONS

(57) Abstract: The present invention relates to a polynucleotide of the EDN/EDNR/ECE signaling system which is associated with at least one cardiovascular disease. Moreover, the invention relates to genes or vectors comprising the polynucleotides of the invention and to a host cell genetically engineered with the polynucleotide or gene of the invention. Further, the invention relates to methods for producing molecular variant polypeptides or fragments thereof, methods for producing cell capable of expressing a molecular variant polypeptide and to a polypeptide or fragment thereof encoded by the polynucleotide or the gene of the invention or which is obtainable by the method or from the cells produced by the method of the invention. Furthermore, the invention relates to an antibody which binds specifically the polypeptide of the invention. Moreover, the invention relates to a transgenic non-human animal. The invention also relates to a solid support comprising one or a plurality of the above mentioned polynucleotides, genes, vectors, polypeptides, antibodies or host cells. Furthermore, methods of identifying a single nucleotide polymorphism, identifying and obtaining a pro-drug or drug or an inhibitor are also encompassed by the present invention. In addition, the invention relates to methods for producing of a pharmaceutical composition and to methods of diagnosing or disease. Further, the invention relates to a method of detection of the polynucleotide of the invention. Furthermore, comprised by the present invention are a diagnostic and a pharmaceutical composition. Even more, the invention relates to uses of the polynucleotides, genes, vectors, polypeptides or antibodies of the invention. Finally, the invention relates to a diagnostic kit.

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Polymorphisms in human genes of cardiovascular regulators and their use in diagnostic and therapeutic applications

The present invention relates to a polynucleotide of the EDN/EDNR/ECE signaling system which is associated with at least one cardiovascular disease. Moreover, the invention relates to genes or vectors comprising the polynucleotides of the invention and to a host cell genetically engineered with the polynucleotide or gene of the invention. Further, the invention relates to methods for producing molecular variant polypeptides or fragments thereof, methods for producing cell capable of expressing a molecular variant polypeptide and to a polypeptide or fragment thereof encoded by the polynucleotide or the gene of the invention or which is obtainable by the method or from the cells produced by the method of the invention. Furthermore, the invention relates to an antibody which binds specifically the polypeptide of the invention. Moreover, the invention relates to a transgenic non-human animal. The invention also relates to a solid support comprising one or a plurality of the above mentioned polynucleotides, genes, vectors, polypeptides, antibodies or host cells. Furthermore, methods of identifying a single nucleotide polymorphism, identifying and obtaining a pro-drug or drug or an inhibitor are also encompassed by the present invention. In addition, the invention relates to methods for producing of a pharmaceutical composition and to methods of diagnosing a disease. Further, the invention relates to a method of detection of the polynucleotide of the invention. Furthermore, comprised by the present invention are a diagnostic and a pharmaceutical composition. Even more, the invention relates to uses of the polynucleotides, genes, vectors, polypeptides or antibodies of the invention. Finally, the invention relates to a diagnostic kit.

Cardiovascular diseases are one of the major health problems in civilized countries. At least two separate groups of factors are known that affect

cardiovascular diseases (risk factors). One set of factors comprises the metabolism of fatty acids (e.g. Hypercholesterolemia, diabetes). Factors and genes that are involved in this aspect of cardiovascular disease have already extensively been genetically analyzed, e.g. the low-density lipoprotein (LDL) receptor mutations, which cause familial hypercholesterolemia (Russell et al. 1989, Atherosclerosis 9 (1 Suppl): I8-13; Hobbs et al. 1992, Hum. Mutat. 1 (6): 445-466). Another complex of genes/factors covers the regulated function of blood vessels: Genes such as endothelins (EDN 1-3), endothelin converting enzyme (ECE-1) and their receptors (EDNRA/EDNRB), are involved in certain types of hypertension, which is a major risk factor in atherosclerosis/cardiovascular disease. Also the risk factor smoking may be involved in this complex of genes/factors: Smoking is associated with endothelial dysfunction and induction of endothelin release, which results in increased plasma levels of endothelins, especially of EDN-1 (Wright et al. 1999, Eur. Respir. J. 14 (5): 1095-1099; Loennechen et al. 1999, Pharmacol. Toxicol. 85 (4): 192-7). Nicotine, affects bFGF mediated regulation of endothelial cells in blood vessels, and may have a key role in the development and progression of atherosclerosis (Cucina et al. 1999, Biochem. Biophys. Res. Commun. 257 (2): 306-312).

Furthermore the genes, which regulate the function of blood vessel walls also play a role in the angiogenesis of tumor growth (Alanen et al. 2000, Histopathology 36 (2): 161-7; Dawas et al. 1999, Ann R Coll Surg Engl 81 (5): 306-310). For tumor therapy different therapeutic approaches regarding the inhibition of angiogenesis will be presently discussed. For example a number of synthetic inhibitors against matrix metalloproteinases (MMPs) have been developed for clinical use (Yip et al. 1999, Invest New Drugs 17 (4): 387-399). Other approaches deal with Adenovirus-mediated gene transfer of endostatin, which results in reduction of tumor growth rates (Sauter et al. 2000, Proc Natl Acad Sci USA 97 (9): 4802-4807). Because of their growth stimulating role in various cancers, the endothelin genes, like EDN-1 are also very promising targets for therapeutic approaches in cancer therapy. Experimental data show, that synthetic retinoids can reduce EDN-1 expression and subsequently lead to growth inhibition of prostate cancer cells (Hsu and Pfahl, 1998, Cancer Res 58 (21): 4817-22).

The human endothelins represent a family comprised of three related vasoactive

isopeptides, endothelin-1, -2 and -3, each of them containing 21 amino acids. They exert mitogenic effects on cells of the vascular system, like endothelial cells, as well as various other cell types via specific G protein-coupled transmembrane receptors (Rubanyi and Polokoff 1994, Pharmacol Review 46: 325-415; Battistini et al. 1993, Peptides 14: 385-399). Each of these peptides has a vasoconstrictor function, but EDN-1 is regarded as the most potent endogenous vasoconstrictor known at present (Yanagisawa et al. 1988, Nature 332: 411-415). Based on clinical and experimental data, a pathophysiological role in human cardiovascular diseases, such as hypertension and atherosclerosis has been postulated for endothelins (Levin ER 1995, N. Engl. J. Med. 333: 356-363). The active endothelin peptides are formed by proteolytic cleavage of inactive precursors, the prepro-endothelin and the "big-endothelin". To generate in a first step the intermediate form, big-endothelin, prepro-endothelin is cleaved by furin (Laporte et al. 1993, J. Cardiovasc. Pharmacol. 22 (Suppl 8): 7-10). The second step is catalyzed in vitro and in vivo by the endothelin converting enzymes (ECE-1), membrane-associated metallopeptidases. There exists homologous proteins, from which ECE-1 may play a key role in the activation and regulation of the cardiovascular endothelin proteolytic cascade. The enzyme can be inhibited in its function by administration of the fungal metabolite phosporamidon (Turner and Murphy 1996, Biochem. Pharmacol 51 (2): 91-102). ECE-1 is expressed in four isoforms with different tissue distribution. These isoforms are generated from the same gene via two promoters, one upstream of exon 1 and the other upstream from exon 3 (Orzechowski et al. 1997, J. Mol. Med. 75: 512-521; Funke-Kaiser et al. 2000, FEBS Lett. 466 (2-3): 310-316).

The biologic effects of endothelins are mediated by the endothelin receptor A (EDNRA), an EDN-1-specific receptor and by the endothelin receptor B (EDNRB), an endothelin-non-specific receptor (Takayanagi et al. 1991, Regul. Pept. 32: 23-37). Different peptide and non-peptide antagonists for the two receptors have been synthesized, e.g. the cyclic pentapeptide BQ-123 for EDNRA and the peptide antagonist BQ-788 for EDNRB (Ihara et al. 1992, Life Sci. 50: 247-255; Ishikawa et al. 1994, Proc. Natl. Acad. Sci. USA 91: 4892-4896). It could be demonstrated, that an anti-endothelin therapy via administration of receptor antagonists, like bosentan (Veniant et al. 1994, Life Sci. 55 (6): 445-454) have a blood-pressure-lowering

effect (Krum et al. 1998, N. Engl. J. Med. 338: 784-790). The two receptors differ in their expression pattern, EDNRB is predominantly expressed on endothelial cells, while EDNRA is present on smooth muscle cells. There exists a relationship between the localisation of expression and the effect of the respective endothelin, which is mediated by one of the two receptors. For example the vasoconstrictive effect of EDN-1 is mediated by the EDNRA receptor, on smooth muscle cells (Sakurai et al. 1990, Nature 348: 732-735; Arai et al. 1990, Nature 348: 730-732; Clozel et al. 1992, Biochem. Biophys. Res. Commun. 186: 867-873).

Because of the roles of the endothelins, their receptors and of the endothelin converting enzyme, genetic variations in their genes may have effects on their function. These effects play a role in the development of cardiovascular diseases and in angiogenesis of tumor growth. In the literature there exist some data regarding a correlation between genetic variants in these genes and atherosclerosis/cardiovascular disease and/or their risk factors, like hypertension are rare. For example Sharma and colleagues have recently published an association between a polymorphism in the 3'-UTR of the EDN-2 gene and human essential hypertension (Sharma et al. 1999, J. Hypertens. 17 (9): 1281-1287). Beyond it sporadic data about associations between genetic variations in the EDN-1 and in the EDNRA gene and blood pressure exist (Nicaud et al. 1999, Am. J. Hypertens. 12 (3): 304-310; Tiret et al. 1999, Hypertension 33 (5): 1169-1174).

Furthermore different experimental data are published, which refer to altered expression levels in correlation to clinical phenotypes. EDN-1 expression has been shown to be enhanced in atherosclerosis and coronary endothelial dysfunction and it may contribute to the rupture of active atherosclerotic plaques, leading to acute ischemic events (Lerman et al. 1995, Circulation 92:2426-2431; Zeiher et al. 1994, Lancet 344: 1405-1406). Moreover elevated expression levels of EDN-1 and -2 could be detected in hypertensive patients and of the endothelin converting enzyme in atherosclerotic lesions (Baldys-Waligorska and Szybinski 1993, Endocr. Regul. 27 (2): 83-87; Minamino et al. 1997: Circulation 95 (1): 221-230). It has to be clarified, if definite SNP's in these genes are the cause of the elevated expressions levels.

All these published data confirm an involvement of the different "endothelin" target genes in clinical phenotypes as hypertension, which leads to atherosclerosis and

subsequently to cardiovascular disease.

Thus, means and methods for diagnosing and treating a variety of cardiovascular diseases and diseases related to dysfunctions of cardiovascular regulators such as the members of the EDN/EDNR/ECE signaling system were not available yet but are nevertheless highly desirable. Thus, the technical problem underlying the present invention is to comply with the above specified needs.

The solution to this technical problem is achieved by providing the embodiments characterized in the claims.

Accordingly, the present invention relates to a polynucleotide comprising a polynucleotide which is associated with at least one cardiovascular disease selected from the group consisting of :

(a) (i) a polynucleotide having the nucleic acid sequence of SEQ ID NO: 77, 78, 83, 84, 89, 90, 95, 96, 101 or 102;

(ii) a polynucleotide being capable of hybridizing to the EDN-1 gene, wherein said polynucleotide is having at a position corresponding to positions 3980 of the EDN-1 gene (Accession No: J05008) a C, at a position corresponding to position 5783 of the EDN-1 gene (Accession No: J05008) a G, at a position corresponding to position 9174 of the EDN-1 gene (Accession No: J05008) a TT, at a position corresponding to positions 10045 of the EDN-1 gene (Accession No: J05008) a C or at a position corresponding to position 10092 of the EDN-1 gene (Accession No: J05008) a C;

(b) (i) a polynucleotide having the nucleic acid sequence of SEQ ID NO: 107, 108, 113, 114, 119, 120, 125, 126, 131, 132, 137, 138, 143 or 144;

(ii) a polynucleotide being capable of hybridizing to the EDN-2 gene, wherein said polynucleotide is having at a position corresponding to position 57 of SEQ ID NO: 415 a T, at a position corresponding to position 32 of SEQ ID NO: 415 a G, at a position corresponding to position 181 of SEQ ID NO: 415 a A, at a position corresponding to

position 133 of SEQ ID NO: 416 a A, at a position corresponding to position 190 of SEQ ID NO: 417 a C, at a position corresponding to position 428 of SEQ ID NO: 418 a C or at a position corresponding to position 287 of SEQ ID NO: 419 a A;

(c) (i) a polynucleotide having the nucleic acid sequence of SEQ ID NO:149, 150, 155, 156, 161 or 162;

(ii) a polynucleotide being capable of hybridizing to the EDN-3 gene, wherein said polynucleotide is having at a position corresponding to position 40215 of the EDN-3 gene (Accession No: AL035250) a A, at a position corresponding to position 59430 of the EDN-3 gene (Accession No: AL035250) a C or at a position corresponding to position 63843 of the EDN-3 gene (Accession No: AL035250) a T;

(d) (i) a polynucleotide having the nucleic acid sequence of SEQ ID NO: 167, 168, 173, 174;

(ii) a polynucleotide being capable of hybridizing to the EDNRA gene, wherein said polynucleotide is having at a position corresponding to position 1366 of the EDNRA gene (Accession No: D11151) a deletion or at a position corresponding to position 1830 of the EDNRA gene (Accession No: D11151) a G;

(e) (i) a polynucleotide having the nucleic acid sequence of SEQ ID NO: 179, 180, 185, 186, 191, 192, 197, 198, 203, 204, 209, 210, 215, 216, 221, 222, 227 or 228;

(ii) a polynucleotide being capable of hybridizing to the EDNRB gene, wherein said polynucleotide is having at a position corresponding to position 749 of the EDNRB gene (Accession No: D13162) a G, at a position corresponding to position 937 of the EDNRB gene (Accession No: D13162) a C, at a position corresponding to position 1112 of the EDNRB gene (Accession No: D13162) a G, at a position corresponding to position 189 of SEQ ID NO: 420 a C, at a position corresponding to position 182 of SEQ ID NO: 421 a G, at a position corresponding to position 1048 of the EDNRB gene (Accession No: D13168) a A, at a position corresponding to position 1658 of the EDNRB gene (Accession No: D13168) a C, at a position

corresponding to position 1912 of the EDNRB gene (Accession No: D13168) a T or at a position corresponding to position 2130 of the EDNRB gene (Accession No: D13168) a T;

(f) (i) a polynucleotide having the nucleic acid sequence of SEQ ID NO: 233, 234, 239, 240, 245, 246, 251, 252, 257, 258, 263, 264, 269, 270, 275, 276, 281, 282, 287, 288, 293, 294, 299, 300, 305, 306, 311, 312, 317, 318, 323, 324, 329, 330, 335, 336, 341, 342, 347, 348, 353, 354, 359, 360, 365, 366, 371, 372, 377, 378, 383, 384, 389, 390, 395, 396, 401, 402 or 422;

(ii) a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 423;

(iii) a polynucleotide being capable of hybridizing to the ECE-1 gene, wherein said polynucleotide is having at a position corresponding to position 86513 of the ECE-1 gene (Accession No: AL031005) a T, at a position corresponding to position 85627 of the ECE-1 gene (Accession No: AL031005) a A, at a position corresponding to position 85424 of the ECE-1 gene (Accession No: AL031005) a T, at a position corresponding to position 85472 of the ECE-1 gene (Accession No: AL031005) a G, at a position corresponding to position 83847 of the ECE-1 gene (Accession No: AL031005) a C, at a position corresponding to position 83924 of the ECE-1 gene (Accession No: AL031005) a T, at a position corresponding to position 75099 of the ECE-1 gene (Accession No: AL031005) a C, at a position corresponding to position 75102 of the ECE-1 gene (Accession No: AL031005) a T, at a position corresponding to position 75354 of the ECE-1 gene (Accession No: AL031005) a A, at a position corresponding to position 75431 of the ECE-1 gene (Accession No: AL031005) a deletion, at a position corresponding to position 73100 of the ECE-1 gene (Accession No: AL031005) a A, at a position corresponding to position 72974 of the ECE-1 gene (Accession No: AL031005) a A, at a position corresponding to position 65946 of the ECE-1 gene (Accession No: AL031005) a T, at a position corresponding to position 65875 of the ECE-1 gene

(Accession No: AL031005) a T, at a position corresponding to position 61579 of the ECE-1 gene (Accession No: AL031005) a G, at a position corresponding to position 61752 of the ECE-1 gene (Accession No: AL031005) a C, at a position corresponding to position 56072 of the ECE-1 gene (Accession No: AL031005) a T, at a position corresponding to position 55860 of the ECE-1 gene (Accession No: AL031005) a A, at a position corresponding to position 55222 of the ECE-1 gene (Accession No: AL031005) a T, at a position corresponding to position 53127 of the ECE-1 gene (Accession No: AL031005) a A, at a position corresponding to position 53217 of the ECE-1 gene (Accession No: AL031005) a T, at a position corresponding to position 53220 of the ECE-1 gene (Accession No: AL031005) a A, at a position corresponding to position 47813 of the ECE-1 gene (Accession No: AL031005) a T, at a position corresponding to position 8625 of the ECE-1 gene (Accession No: AL031728) a T, at a position corresponding to position 8938 of the ECE-1 gene (Accession No: AL031728) a T, at a position corresponding to position 9351 of the ECE-1 gene (Accession No: AL031728) a G, at a position corresponding to position 9439 of the ECE-1 gene (Accession No: AL031728) a C, at a position corresponding to position 109728 of the ECE-1 gene (Accession No: AL031005) a A, at a position corresponding to position 109249 of the ECE-1 gene (Accession No: AL031005) a T or at a position corresponding to position 1008 of the ECE-1 gene (Accession No: Z35307) a T:;

(iv) a polynucleotide encoding an ECE-1 polypeptide or fragment thereof, wherein said polypeptide comprises an amino acid substitution of Thr to Ile at position 324 of the sequence of the ECE-1 polypeptide (Accession No: CAA84548).

In the context of the present invention the term "polynucleotides" or the term "polypeptides" refers to different variants of a polynucleotide or polypeptide. Said variants comprise a reference or wild type sequence of the polynucleotides or

polypeptides of the invention as well as variants which differ therefrom in structure or composition. Reference or wild type sequences for the polynucleotides are Accession No: J05008 for EDN-1, Accession No: M65199 for EDN-2, Accession No: AL035250 for EDN-3, Accession No: D11151 or X61950 for EDNRA, Accession No: D13162, D13168 or D90402 for EDNRB and Accession No: AL031005, AL031728 or Z35307 for ECE-1. Reference or wild type sequences for the polypeptides of the invention are Accession No: 1311366 or NP_001946 for EDN-1, Accession No: P20800 for EDN-2, Accession No: NP_000105 for EDN-3, Accession No: NP_001948 for EDNRA, Accession No: NP_000106 for EDNRB and Accession No: CAA84548 for ECE-1. The differences in structure or composition usually occur by way of nucleotide or amino acid substitution(s), addition(s) and/or deletion(s). Preferably, said nucleotide substitution(s), addition(s) or deletion(s) result(s) in one or more changes of the corresponding amino acid(s) of the polypeptides of the invention.

The term "hybridizing" as used herein refers to polynucleotides which are capable of hybridizing to the polynucleotides of the invention or parts thereof which are associated with a dysfunction or dysregulation of the EDN/EDNR/ECE signalling system which are related to at least one of the cardiovascular diseases or a susceptibility therefor referred to in this specification. Thus, said hybridizing polynucleotides are also associated with said dysfunctions and dysregulations. Preferably, said polynucleotides capable of hybridizing to the polynucleotides of the invention or parts thereof which are associated with dysfunctions or dysregulations of the EDN/EDNR/ECE signalling system are at least 70%, at least 80%, at least 95% or at least 100% identical to the polynucleotides of the invention or parts thereof which are associated with said dysfunctions or dysregulations. Therefore, said polynucleotides are useful, e.g. as probes in Northern or Southern Blot analysis of RNA or DNA preparations, respectively, or can be used as oligonucleotide primers in PCR analysis dependent on their respective size. Also comprised by the invention are hybridizing polynucleotides which are useful for analysing DNA-Protein interactions via, e.g., electrophoretic mobility shift analysis (EMSA). Preferably, said hybridizing polynucleotides comprise at least 10, more preferably at least 15 nucleotides in length while a hybridizing polynucleotide of the

present invention to be used as a probe preferably comprises at least 100, more preferably at least 200, or most preferably at least 500 nucleotides in length.
It is well known in the art how to perform hybridization experiments with nucleic acid molecules, i.e. the person skilled in the art knows what hybridization conditions s/he has to use in accordance with the present invention. Such hybridization conditions are referred to in standard text books such as Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory (1989) N.Y. Preferred in accordance with the present inventions are polynucleotides which are capable of hybridizing to the polynucleotides of the invention or parts thereof which are associated with a dysfunction or dysregulation of the EDN/EDNR/ECE signalling system under stringent hybridization conditions, i.e. which do not cross hybridize to unrelated polynucleotides such as polynucleotides encoding a polypeptide different from the polypeptides of the invention.

The term "corresponding" as used herein means that a position is not only determined by the number of the preceding nucleotides and amino acids, respectively. The position of a given nucleotide or amino acid in accordance with the present invention which may be deleted, substituted or comprise one or more additional nucleotide(s) may vary due to deletions or additional nucleotides or amino acids elsewhere in the gene or the polypeptide. Thus, under a "corresponding position" in accordance with the present invention it is to be understood that nucleotides or amino acids may differ in the indicated number but may still have similar neighboring nucleotides or amino acids. Said nucleotides or amino acids which may be exchanged, deleted or comprise additional nucleotides or amino acids are also comprised by the term "corresponding position". Said nucleotides or amino acids may for instance together with their neighbors form sequences which may be involved in the regulation of gene expression, stability of the corresponding RNA or RNA editing, as well as encode functional domains or motifs of the protein of the invention.

In accordance with the present invention, the mode and population distribution of novel so far unidentified genetic variations in the genes for members of the EDN/EDNR/ECE signaling system have been analyzed by sequence analysis of

relevant regions of the human said genes from many different individuals. It is a well known fact that genomic DNA of individuals, which harbor the individual genetic makeup of all genes, including genes for members of the EDN/EDNR/ECE signaling system can easily be purified from individual blood samples. These individual DNA samples are then used for the analysis of the sequence composition of the alleles of genes for members of the EDN/EDNR/ECE signaling system that are present in the individual which provided the blood sample. The sequence analysis was carried out by PCR amplification of relevant regions of the said genes, subsequent purification of the PCR products, followed by automated DNA sequencing with established methods (e.g. ABI dyeterminator cycle sequencing).

One important parameter that had to be considered in the attempt to determine the individual genotypes and identify novel variants of genes for members of the EDN/EDNR/ECE signaling system by direct DNA-sequencing of PCR-products from human blood genomic DNA is the fact that each human harbors (usually, with very few abnormal exceptions) two gene copies of each autosomal gene (diploidy). Because of that, great care had to be taken in the evaluation of the sequences to be able to identify unambiguously not only homozygous sequence variations but also heterozygous variations. The details of the different steps in the identification and characterization of novel polymorphisms in genes for members of the EDN/EDNR/ECE signaling system (homozygous and heterozygous) are described in the Examples 1 to 3 below.

The mutations in the genes for members of the EDN/EDNR/ECE signaling system detected in accordance with the present invention are listed in Table 2. The methods of the mutation analysis followed standard protocols and are described in detail in the Examples. In general such methods are to be used in accordance with the present invention for evaluating the phenotypic spectrum as well as the overlapping clinical characteristics of diseases or conditions related to cardiovascular deficiencies, such as cardiovascular diseases and/or diseases related to abnormal angiogenesis. Advantageously, the characterization of said mutants may form the basis of the development of improved drugs, such as drugs which are used in cancer therapy or regulation of angiogenesis in patients with

mutations in the genes for members of the EDN/EDNR/ECE signaling system. Said methods encompass for example haplotype analysis, single-strand conformation polymorphism analysis (SSCA), PCR and direct sequencing; see also Cambien (1999), and references cited therein. On the basis of thorough clinical characterization of many patients the phenotypes can then be correlated to these mutations as well as to mutations that had been described earlier, for example in Cambien (1999).

As is evident to the person skilled in the art this new molecular genetic knowledge can now be used to exactly characterize the genotype of the index patient where a given drug takes an unusual effect and of his family.

Over the past 20 years, genetic heterogeneity has been increasingly recognized as a significant source of variation in drug response. Many scientific communications (Meyer, Ann. Rev. Pharmacol. Toxicol. 37 (1997), 269-296 and West, J. Clin. Pharmacol. 37 (1997), 635-648) have clearly shown that some drugs work better or may even be highly toxic in some patients than in others and that these variations in patient's responses to drugs can be related to molecular basis. This "pharmacogenomic" concept spots correlations between responses to drugs and genetic profiles of patient's (Marshall, Nature Biotechnology, 15 (1997), 954-957; Marshall, Nature Biotechnology, 15 (1997), 1249-1252).

In this context of population variability with regard to drug therapy, pharmacogenomics has been proposed as a tool useful in the identification and selection of patients which can respond to a particular drug without side effects. This identification/selection can be based upon molecular diagnosis of genetic polymorphisms by genotyping DNA from leukocytes in the blood of patient, for example, and characterization of disease (Bertz, Clin. Pharmacokinet. 32 (1997), 210-256; Engel, J. Chromatogra. B. Biomed. Appl. 678 (1996), 93-103). For the founders of health care, such as health maintenance organizations in the US and government public health services in many European countries, this pharmacogenomics approach can represent a way of both improving health care

and reducing overheads because there is a large cost to unnecessary drugs, ineffective drugs and drugs with side effects.

The mutations in the variant genes of the invention sometime result in amino acid deletion(s), insertion(s) and in particular in substitution(s) either alone or in combination. It is of course also possible to genetically engineer such mutations in wild type genes or other mutant forms. Methods for introducing such modifications in the DNA sequence of said genes are well known to the person skilled in the art; see, e.g., Sambrook, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory (1989) N.Y.

For the investigation of the nature of the alterations in the amino acid sequence of the polypeptides of the EDN/EDNR/ECE signaling system computer programs may be used such as BRASMOL that are obtainable from the Internet. Furthermore, folding simulations and computer redesign of structural motifs can be performed using other appropriate computer programs (Olszewski, Proteins 25 (1996), 286-299; Hoffman, Comput. Appl. Biosci. 11 (1995), 675-679). Computers can be used for the conformational and energetic analysis of detailed protein models (Monge, J. Mol. Biol. 247 (1995), 995-1012; Renouf, Adv. Exp. Med. Biol. 376 (1995), 37-45). These analysis can be used for the identification of the influence of a particular mutation on binding and/or processing of drugs.

Usually, said amino acid deletion, addition or substitution in the amino acid sequence of the protein encoded by the polynucleotide of the invention is due to one or more nucleotide substitution, insertion or deletion, or any combinations thereof. Preferably said nucleotide substitution, insertion or deletion may result in an amino acid substitution of Thr324 to Ile of the ECE-1 polypeptide.

The polynucleotide of the invention may further comprise at least one nucleotide and optionally amino acid deletion, addition and/or substitution other than those specified hereinabove, for example those described in the prior art; e.g., Cambien (1999). This embodiment of the present invention allows the study of synergistic effects of the mutations in the genes for members of the EDN/EDNR/ECE signaling

system on the pharmacological profile of drugs in patients who bear such mutant forms of the gene or similar mutant forms that can be mimicked by the above described proteins. It is expected that the analysis of said synergistic effects provides deeper insights into the onset of cardiovascular deficiencies as described supra. From said deeper insight the development of diagnostic and pharmaceutical compositions related to cardiovascular deficiencies such as cancer or abnormal angiogenesis will greatly benefit.

Finally, the polynucleotides and polypeptides referred to in accordance with the present invention are also useful as forensic markers, which improve the identification of subjects which have been murdered or killed by, for example, a crime of violence or any other violence and can not be identified by the well known conventional forensic methods. The application of forensic methods based on the detection of the polymorphisms comprised by the polynucleotides of this invention in the genome of a subject are particularly well suited in cases where a (dead) body is disfigured in a severe manner such that identification by other body characteristics such as the features of the face is not possible. This is the case, for example, for corpse found in water which are usually entirely disfigured. Advantageously methods which are based on the provision of the polynucleotides of the invention merely require a minimal amount of tissues or cells in order to be carried out. Said tissues or cells may be blood droplets, hair roots, epidermal scales, saliva droplets, sperms etc. Since only such a minimal amount of tissues or cells are required for the identification of a subject, the polymorphisms comprised by the polynucleotides of this invention can be also used as forensic markers in order to prove someone guilt of a crime, such as a violation or a ravishment. Moreover, the polymorphisms comprised by the polynucleotides of this invention can be used to proof paternity. In accordance with the forensic methods referred to herein the presence or absence of the polynucleotides of the invention is determined and compared with a reference sample which is unambiguously derived from the subject to be identified. The forensic methods which require detection of the presence or absence of the polynucleotides of the invention in a sample of a subject the polymorphisms comprised by the polynucleotides of this invention can be for example PCR-based techniques which are particularly well

suited in cases where only a minimal amount of tissues or cells are available as forensic samples. On the other hand, where enough tissue or cells are available, hybridization based techniques may be performed in order to detect the presence or absence of a polynucleotide of this invention. These techniques are well known by the person skilled in the art and can be adopted to the individual purposes referred to herein without further ado. In conclusion, thanks to the present invention forensic means which allow improved and reliable predictions as regards the aforementioned aspects are now available.

In a preferred embodiment of the present invention said cardiovascular disease is a coronary heart disease, hypertension, atherosclerosis or related to abnormal angiogenesis.

The above mentioned diseases are very well known and characterized for the person skilled in the art. For a detailed list of symptoms which are indicative for said diseases it is referred to text book knowledge, e.g. Pschyrembel or e.g. in the field of angiogenesis: Tomanek and Schatteman 2000, Anat. Rec.261 (3): 126-35, Griffioen and Molema 2000, Pharmacol. Rev. 52 (2): 237-268 and Fidler 2000, Cancer J. Sci. Am. Suppl. 2: 134-141 or in the field of cardiovascular diseases and risk factors, like hypertension: Rüscher and Barton 1997, Clin. Cardiol. 20, Suppl. II, II-3 – II-10, Panza 1997, Clin. Cardiol. 20, Suppl. II, II-26 – II-33, Cooke 1997, Clin. Cardiol. 20, Suppl. II, II-45 – II-51 and Pepine 1997, Clin. Cardiol. 20, Suppl. II, II-58 – II-64.

In another preferred embodiment of the present invention the encoded polypeptide is a member of the EDN/EDNR/ECE signaling system.

The polypeptides referred to in the present invention constitute a signaling system. The ECE-1 polypeptide is required to activate the intercellular signal polypeptides EDN-1, -2 or -3 before binding to their respective receptors, the EDNRA/B polypeptides. Numerous of said signaling system have been described already. Said signaling system from the basis for intercellular communication which is a prerequisite for constructing and maintaining an organism physiologically and

morphologically. It is very well know in the art that all components of said signaling system are required together to constitute a functional signaling system. Usually each component is equally important in this respect. Therefore, a single point mutation which may influence the function of an individual component of said signaling system should also influence the function of the signaling system in its entirety. For the present invention single nucleotide polymorphisms in a component of the EDN/EDNR/ECE signaling system as described above may result in an altered or in loss of function of the entire EDN/EDNR/ECE signaling system and should therefore elict similar deficiencies.

As described supra, the EDN/EDNR/ECE signaling system is involved in the formation and regulation of the cardiovascular system. Thus, single nucleotide polymorphisms in any one of the components of the EDN/EDNR/ECE signaling system may be indicative, e.g., for cardiovascular deficiencies.

In a further embodiment the present invention relates to a polynucleotide which is DNA or RNA.

The polynucleotide of the invention may be, e.g., DNA, cDNA, genomic DNA, RNA or synthetically produced DNA or RNA or a recombinantly produced chimeric nucleic acid molecule comprising any of those polynucleotides either alone or in combination. Preferably said polynucleotide is part of a vector, particularly plasmids, cosmids, viruses and bacteriophages used conventionally in genetic engineering that comprise a polynucleotide of the invention. Such vectors may comprise further genes such as marker genes which allow for the selection of said vector in a suitable host cell and under suitable conditions.

The invention furthermore relates to a gene comprising the polynucleotide of the invention.

It is well known in the art that genes comprise structural elements which encode an amino acid sequence as well as regulatory elements which are involved in the regulation of the expression of said genes. Structural elements may be

represented by exons which may either encode an amino acid sequence or which may encode for RNA which is not encoding an amino acid sequence but is nevertheless involved in RNA function, e.g. by regulating the stability of the RNA or the nuclear expert of the RNA.

Regulatory elements of a gene may comprise promoter elements or enhancer elements both of which could be involved in transcriptional control of gene expression. It is very well known in the art that a promoter is to be found upstream of the structural elements of a gene. Regulatory elements such as enhancer elements, however, may be found distributed over the entire locus of gene. Said elements could be reside, e.g., in introns, regions of genomic DNA which separate the exons of a gene. Said introns may comprise further regulatory elements which are required for proper gene expression. Introns are usually transcribed together with the exons of a gene resulting in a nascent RNA transcript which contains both, exon and intron sequences. The intron encoded RNA sequences are usually removed by a process known as RNA splicing. However, said process also requires regulatory sequences present on a RNA transcript which may be encoded by the introns.

In addition, besides their function in transcriptional control and control of proper RNA processing and/or stability, regulatory elements of a gene might be also involved in the control of genetic stability of a gene locus. Said elements may control, e.g., recombination events or may serve to maintain a certain structure of the DNA or the arrangement of DNA in a chromosome.

Therefore, single nucleotide polymorphisms may occur in exons of a gene which encode an amino acid sequence as discussed supra as well as in regulatory regions which are involved in the above discussed process. The provision of the nucleotide sequence of a gene locus in its entirety including, e.g., introns is in light of the above desirable with respect to the analysis and the determination of new single nucleotide polymorphisms and therefore comprised by the present invention.

In a preferred embodiment of the invention said gene is further comprising any one

of the polynucleotides having the nucleic acid sequence of any one of SEQ ID NO: 403 to 410 or any one of SEQ ID NO: 411 to 414.

In a furthermore preferred embodiment the invention relates to a gene, wherein a nucleotide deletion, addition and/or substitution results in altered expression of the variant gene compared to the corresponding wild type gene.

In another embodiment the present invention relates to a vector comprising the polynucleotide of the invention or the gene of the invention.

Said vector may be, for example, a phage, plasmid, viral or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host/cells. The polynucleotides or genes of the invention may be joined to a vector containing a selectable markers for propagation in a host. Generally, a plasmid vector is introduced in a precignitate such as a calcium phosphate precignitate, or in a complex with a charged lipid or in carbon-based clusters. Should the vector be a virus, it may be packaged in vitro using an appropriate packaging cell line prior to application to host cells.

In a more preferred embodiment of the vector of the invention the polynucleotide is operatively linked to expression control sequences allowing expression in prokaryotic or eukaryotic cells or isolated fractions thereof.

Expression of said polynucleotide comprises transcription of the polynucleotide, preferably into a translatable mRNA. Regulatory elements ensuring expression in eukaryotic cells, preferably mammalian cells, are well known to those skilled in the art. They usually comprise regulatory sequences ensuring initiation of transcription and optionally poly-A signals ensuring termination of transcription and stabilization of the transcript. Additional regulatory elements may include transcriptional as well as translational enhancers. Possible regulatory elements permitting expression in prokaryotic host cells comprise, e.g., the *lac*, *trp* or *tac* promoter in *E. coli*, and examples for regulatory elements permitting expression in eukaryotic host cells are

the *AOX1* or *GAL1* promoter in yeast or the CMV-, SV40- , RSV-promoter (Rous sarcoma virus), CMV-enhancer, SV40-enhancer or a globin intron in mammalian and other animal cells. Beside elements which are responsible for the initiation of transcription such regulatory elements may also comprise transcription termination signals, such as the SV40-poly-A site or the tk-poly-A site, downstream of the polynucleotide. In this context, suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pCDM8, pRc/CMV, pcDNA1, pcDNA3 (In-vitrogene), pSPORT1 (GIBCO BRL). Preferably, said vector is an expression vector and/or a gene transfer or targeting vector. Expression vectors derived from viruses such as retroviruses, vaccinia virus, adeno-associated virus, herpes viruses, or bovine papilloma virus, may be used for delivery of the polynucleotides or vector of the invention into targeted cell population. Methods which are well known to those skilled in the art can be used to construct recombinant viral vectors; see, for example, the techniques described in Sambrook, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory (1989) N.Y. and Ausubel, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y. (1994). Alternatively, the polynucleotides and vectors of the invention can be reconstituted into liposomes for delivery to target cells.

The present invention furthermore relates to a host cell genetically engineered with the polynucleotide of the invention, the gene of the invention or the vector of the invention.

Said host cell may be a prokaryotic or eukaryotic cell; see supra. The polynucleotide or vector of the invention which is present in the host cell may either be integrated into the genome of the host cell or it may be maintained extrachromosomally. In this respect, it is also to be understood that the recombinant DNA molecule of the invention can be used for "gene targeting" and/or "gene replacement", for restoring a mutant gene or for creating a mutant gene via homologous recombination; see for example Mouellic, Proc. Natl. Acad. Sci. USA, 87 (1990), 4712-4716; Joyner, Gene Targeting, A Practical Approach, Oxford University Press.

The host cell can be any prokaryotic or eukaryotic cell, such as a bacterial, insect, fungal, plant, animal or human cell. Preferred fungal cells are, for example, those of the genus Saccharomyces, in particular those of the species S. cerevisiae. The term "prokaryotic" is meant to include all bacteria which can be transformed or transfected with a polynucleotide for the expression of a variant polypeptide of the EDN/EDNR/ECE signaling system or fragment thereof. Prokaryotic hosts may include gram negative as well as gram positive bacteria such as, for example, *E. coli*, *S. typhimurium*, *Serratia marcescens* and *Bacillus subtilis*. A polynucleotide coding for a mutant form of variant polypeptides of the EDN/EDNR/ECE signaling system can be used to transform or transfect the host using any of the techniques commonly known to those of ordinary skill in the art. Methods for preparing fused, operably linked genes and expressing them in bacteria or animal cells are well-known in the art (Sambrook, supra). The genetic constructs and methods described therein can be utilized for expression of variant polypeptides of the EDN/EDNR/ECE signaling system in, e.g., prokaryotic hosts. In general, expression vectors containing promoter sequences which facilitate the efficient transcription of the inserted polynucleotide are used in connection with the host. The expression vector typically contains an origin of replication, a promoter, and a terminator, as well as specific genes which are capable of providing phenotypic selection of the transformed cells. The transformed prokaryotic hosts can be grown in fermentors and cultured according to techniques known in the art to achieve optimal cell growth. The proteins of the invention can then be isolated from the grown medium, cellular lysates, or cellular membrane fractions. The isolation and purification of the microbially or otherwise expressed polypeptides of the invention may be by any conventional means such as, for example, preparative chromatographic separations and immunological separations such as those involving the use of monoclonal or polyclonal antibodies.

Thus, in a further embodiment the invention relates to a method for producing a molecular variant polypeptide or fragment thereof which is associated with at least one cardiovascular disease comprising culturing the above described host cell; and recovering said protein or fragment from the culture.

In another embodiment the present invention relates to a method for producing cells capable of expressing a molecular variant polypeptide which is associated with at least one cardiovascular disease comprising genetically engineering cells with the polynucleotide of the invention, the gene of the invention or the vector of the invention.

The cells obtainable by the method of the invention can be used, for example, to test drugs according to the methods described in D. L. Spector, R. D. Goldman, L. A. Leinwand, Cells, a Lab manual, CSH Press 1998. Furthermore, the cells can be used to study known drugs and unknown derivatives thereof for their ability to complement the deficiency caused by mutations in the genes for members of the EDN/EDNR/ECE signaling system. For these embodiments the host cells preferably lack a wild type allele, preferably both alleles of the genes for members of the EDN/EDNR/ECE signaling system and/or have at least one mutated from thereof. Alternatively, strong overexpression of a mutated allele over the normal allele and comparison with a recombinant cell line overexpressing the normal allele at a similar level may be used as a screening and analysis system. The cells obtainable by the above-described method may also be used for the screening methods referred to herein below.

Furthermore, the invention relates to a polypeptide or fragment thereof encoded by the polynucleotide of the invention, the gene of the invention or obtainable by the method described above or from cells produced by the method described above.

In this context it is also understood that the variant polypeptides of the EDN/EDNR/ECE signaling system according to the invention may be further modified by conventional methods known in the art. By providing said variant proteins according to the present invention it is also possible to determine the portions relevant for their biological activity or inhibition of the same. The terms "polypeptide" and "protein" as used herein are exchangable. Moreover, what is comprised by said terms is standard textbook knowledge.

The present invention furthermore relates to an antibody which binds specifically to

the polypeptide of the invention.

Advantageously, the antibody specifically recognizes or binds an epitope containing one or more amino acid substitution(s) as defined above. Antibodies against the variant polypeptides of the invention can be prepared by well known methods using a purified protein according to the invention or a (synthetic) fragment derived therefrom as an antigen. Monoclonal antibodies can be prepared, for example, by the techniques as originally described in Köhler and Milstein, Nature 256 (1975), 495, and Galfré, Meth. Enzymol. 73 (1981), 3, which comprise the fusion of mouse myeloma cells to spleen cells derived from immunized mammals. In a preferred embodiment of the invention, said antibody is a monoclonal antibody, a polyclonal antibody, a single chain antibody, human or humanized antibody, primatized, chimerized or fragment thereof that specifically binds said peptide or polypeptide also including bispecific antibody, synthetic antibody, antibody fragment, such as Fab, Fv or scFv fragments etc., or a chemically modified derivative of any of these. Furthermore, antibodies or fragments thereof to the aforementioned polypeptides can be obtained by using methods which are described, e.g., in Harlow and Lane "Antibodies, A Laboratory Manual", CSH Press, Cold Spring Harbor, 1988. These antibodies can be used, for example, for the immunoprecipitation and immunolocalization of the variant polypeptides of the invention as well as for the monitoring of the presence of said variant polypeptides, for example, in recombinant organisms, and for the identification of compounds interacting with the proteins according to the invention. For example, surface plasmon resonance as employed in the BIAcore system can be used to increase the efficiency of phage antibodies which bind to an epitope of the protein of the invention (Schier, Human Antibodies Hybridomas 7 (1996), 97-105; Malmborg, J. Immunol. Methods 183 (1995), 7-13).

In a preferred embodiment the antibody of the present invention specifically recognizes an epitope containing one or more amino acid substitution(s) resulting from a nucleotide exchange as defined supra.

Antibodies which specifically recognize modified amino acids such as phospho-

Tyrosine residues are well known in the art. Similarly, in accordance with the present invention antibodies which specifically recognize even a single amino acid exchange in an epitope may be generated, e.g. by the well known methods described supra.

In a more preferred embodiment the antibody of the present invention is monoclonal or polyclonal.

The invention also relates to a transgenic non-human animal comprising at least one polynucleotide of the invention, the gene of the invention or the vector of the invention as described supra.

The present invention also encompasses a method for the production of a transgenic non-human animal, preferably transgenic mouse, comprising introduction of a polynucleotide or vector of the invention into a germ cell, an embryonic cell, stem cell or an egg or a cell derived therefrom. The non-human animal can be used in accordance with the method of the invention described below and may be a non-transgenic healthy animal, or may have a disease or disorder, preferably a cardiovascular disease caused by at least one mutation in at least one gene of the invention. Such transgenic animals are well suited for, e.g., pharmacological studies of drugs in connection with variant forms of the above described variant polypeptides since these polypeptides or at least their functional domains are conserved between species in higher eukaryotes, particularly in mammals. Production of transgenic embryos and screening of those can be performed, e.g., as described by A. L. Joyner Ed., Gene Targeting, A Practical Approach (1993), Oxford University Press. The DNA of the embryos can be analyzed using, e.g., Southern blots with an appropriate probe or based on PCR techniques.

A transgenic non-human animal in accordance with the invention may be a transgenic mouse, rat, hamster, dog, monkey, rabbit, pig, frog, C. elegans, fruitfly such as Drosophila and fish such as torpedo fish or zebrafish comprising a polynucleotide or vector of the invention or obtained by the method described

above, preferably wherein said polynucleotide or vector is stably integrated into the genome of said non-human animal, preferably such that the presence of said polynucleotide or vector leads to the expression of the variant polypeptide of the invention. It may comprise one or several copies of the same or different polynucleotides or genes of the invention. This animal has numerous utilities, including as a research model for cardiovascular research and therefore, presents a novel and valuable animal in the development of therapies, treatment, etc. for diseases caused by cardiovascular diseases. Accordingly, in this instance, the mammal is preferably a laboratory animal such as a mouse, rat or zebrafish.

In a preferred embodiment of the invention the transgenic non-human animal is a mouse, a rat or a zebrafish.

Numerous reports revealed that said animals are particularly well suited as model organisms for the investigation of the cardiovascular system and its deficiencies. Advantageously, transgenic animals can be easily created using said model organisms.

The invention also relates to a solid support comprising one or a plurality of the polynucleotide, the gene, the vector, the polypeptide, the antibody or the host cell of the invention in immobilized form.

The term "solid support" as used herein refers to a flexible or non-flexible support that is suitable for carrying said immobilized targets. Said solid support may be homogenous or inhomogeneous. For example, said solid support may consist of different materials having the same or different properties with respect to flexibility and immobilization, for instance, or said solid support may consist of one material exhibiting a plurality of properties also comprising flexibility and immobilization properties. Said solid support may comprise glass-, polypropylene- or silicon-chips, membranes oligonucleotide-conjugated beads or bead arrays.

The term "immobilized" means that the molecular species of interest is fixed to a solid support, preferably covalently linked thereto. This covalent linkage can be

achieved by different means depending on the molecular nature of the molecular species. Moreover, the molecular species may be also fixed on the solid support by electrostatic forces, hydrophobic or hydrophilic interactions or Van-der-Waals forces. The above described physico-chemical interactions typically occur in interactions between molecules. For example, biotinylated polypeptides may be fixed on a avidin-coated solid support due to interactions of the above described types. Further, polypeptides such as antibodies, may be fixed on an antibody coated solid support. Moreover, the immobilization is dependent on the chemical properties of the solid support. For example, the nucleic acid molecules can be immobilized on a membrane by standard techniques such as UV-crosslinking or heat.

In a preferred embodiment of the invention said solid support is a membrane, a glass- or poylpropylene- or silicon-chip, are oligonucleotide-conjugated beads or a bead array, which is assembled on an optical filter substrate.

Moreover, the present invention relates to an in vitro method for identifying a single nucleotide polymorphism said method comprising the steps of:

(a) isolating a polynucleotide or the gene of the invention from a plurality of subgroups of individuals, wherein one subgroup has no prevalence for a cardiovascular disease and at least one or more further subgroup(s) do have prevalence for a cardiovascular disease; and

(b) identifying a single nucleotide polymorphism by comparing the nucleic acid sequence of said polynucleotide or said gene of said one subgroup having no prevalence for a cardiovascular disease with said at least one or more further subgroup(s) having a prevalence cardiovascular disease.

The term "prevalence" as used herein means that individuals may be susceptible for one or more cardiovascular disease(s) or could already have one or more cardiovascular disease(s). Hereby, one cardiovascular disease may be used to determine the susceptibility for another cardiovascular disease, e.g. atherosclerosis may be indicative for a prevalence for, e.g. heart insufficiencies. Moreover, symptoms which are indicative for a prevalence of cardiovascular diseases are

very well known in the art and have been sufficiently described in standard textbooks such as Pschyrembel or in the field of cardiovascular diseases and risk factors, like hypertension: Rüscher and Barton 1997, Clin. Cardiol. 20, Suppl. II, II-3 – II-10, Panza 1997, Clin. Cardiol. 20, Suppl. II, II-26 – II-33, Cooke 1997, Clin. Cardiol. 20, Suppl. II, II-45 – II-51 and Pepine 1997, Clin. Cardiol. 20, Suppl. II, II-58 – II-64.

Usually, polymorphisms according to the present invention which are associated with one or more cardiovascular disease(s) should be enriched in subgroups of individuals which have a prevalence for said diseases versus subgroups which have no prevalence for said diseases.
Thus, the above described method allows the rapid and reliable detection of polymorphism which are indicative for one or more cardiovascular disease or a susceptibility therefor. Advantageously, due to the phenotypic preselection a large number of individuals having no prevalence might be screened for polymorphisms in general. Thereby, a reference sequences comprising polymorphisms which do not correlate to one or more cardiovascular disease(s) can be obtained. Based on said reference sequences it is possible to efficiently and reliably determine the relevant polymorphisms which can be correlated with one or more cardiovascular disease(s).

In a further embodiment the present invention relates to a method for identifying and obtaining a pro-drug or a drug capable of modulating the activity of a molecular variant of a polypeptide of the EDN/EDNR/ECE signaling system comprising the steps of:

(a) contacting the polypeptide, the solid support of the invention, a cell expressing a molecular variant gene comprising a polynucleotide of the invention, the gene or the vector of the invention in the presence of components capable of providing a detectable signal in response to drug activity with a compound to be screened for pro-drug or drug activity; and

(b) detecting the presence or absence of a signal or increase or decrease of a signal generated from the pro-drug or the drug activity, wherein the absence, presence, increase or decrease of the signal is indicative for a

putative pro-drug or drug.

The term "compound" in a method of the invention includes a single substance or a plurality of substances which may or may not be identical.

Said compound(s) may be chemically synthesized or produced via microbial fermentation but can also be comprised in, for example, samples, e.g., cell extracts from, e.g., plants, animals or microorganisms. Furthermore, said compounds may be known in the art but hitherto not known to be useful as an inhibitor, respectively. The plurality of compounds may be, e.g., added to the culture medium or injected into a cell or non-human animal of the invention.

If a sample containing (a) compound(s) is identified in the method of the invention, then it is either possible to isolate the compound from the original sample identified as containing the compound, in question or one can further subdivide the original sample, for example, if it consists of a plurality of different compounds, so as to reduce the number of different substances per sample and repeat the method with the subdivisions of the original sample. It can then be determined whether said sample or compound displays the desired properties, for example, by the methods described herein or in the literature (Spector et al., Cells manual; see supra). Depending on the complexity of the samples, the steps described above can be performed several times, preferably until the sample identified according to the method of the invention only comprises a limited number of or only one substance(s). Preferably said sample comprises substances of similar chemical and/or physical properties, and most preferably said substances are identical. The methods of the present invention can be easily performed and designed by the person skilled in the art, for example in accordance with other cell based assays described in the prior art or by using and modifying the methods as described herein. Furthermore, the person skilled in the art will readily recognize which further compounds may be used in order to perform the methods of the invention, for example, enzymes, if necessary, that convert a certain compound into a precursor. Such adaptation of the method of the invention is well within the skill of the person skilled in the art and can be performed without undue experimentation.

Compounds which can be used in accordance with the present invention include peptides, proteins, nucleic acids, antibodies, small organic compounds, ligands, peptidomimetics, PNAs and the like. Said compounds may act as agonists or antagonists of the inveniton. Said compounds can also be functional derivatives or analogues of known drugs. Methods for the preparation of chemical derivatives and analogues are well known to those skilled in the art and are described in, for example, Beilstein, Handbook of Organic Chemistry, Springer edition New York Inc., 175 Fifth Avenue, New York, N.Y. 10010 U.S.A. and Organic Synthesis, Wiley, New York, USA. Furthermore, said derivatives and analogues can be tested for their effects according to methods known in the art or as described. Furthermore, peptide mimetics and/or computer aided design of appropriate drug derivatives and analogues can be used, for example, according to the methods described below. Such analogs comprise molecules may have as the basis structure of known ECE-1 substrates and/or inhibitors and/or modulators; see infra. Further, agonists or antagonists of the EDNR receptors may be closely related to the EDN polypeptides.

Appropriate computer programs can be used for the identification of interactive sites of a putative inhibitor and the polypeptides of the invention by computer assistant searches for complementary structural motifs (Fassina, Immunomethods 5 (1994), 114-120). Further appropriate computer systems for the computer aided design of protein and peptides are described in the prior art, for example, in Berry, Biochem. Soc. Trans. 22 (1994), 1033-1036; Wodak, Ann. N. Y. Acad. Sci. 501 (1987), 1-13; Pabo, Biochemistry 25 (1986), 5987-5991. The results obtained from the above-described computer analysis can be used in combination with the method of the invention for, e.g., optimizing known inhibitors, analogs, antagonists or agonists. Appropriate peptidomimetics and other inhibitors can also be identified by the synthesis of peptidomimetic combinatorial libraries through successive chemical modification and testing the resulting compounds, e.g., according to the methods described herein. Methods for the generation and use of peptidomimetic combinatorial libraries are described in the prior art, for example in Ostresh, Methods in Enzymology 267 (1996), 220-234 and Dorner, Bioorg. Med. Chem. 4 (1996), 709-715. Furthermore, the three-dimensional and/or crystallographic structure of said compounds and the polypeptides of the invention can be used for

the design of peptidomimetic drugs (Rose, Biochemistry 35 (1996), 12933-12944; Rutenber, Bioorg. Med. Chem. 4 (1996), 1545-1558). It is very well known how to obtain said compounds, e.g. by chemical or biochemical standard techniques. Thus, also comprised by the method of the invention are means of making or producing said compounds. In summary, the present invention provides methods for identifying and obtaining compounds which can be used in specific doses for the treatment of specific forms of diseases, e.g. cardiovascular diseases or abnormal angiogenesis or cancer.

The above definitions apply mutatis mutandis to all of the methods described in the following.

In a further embodiment the present invention relates to a method for identifying and obtaining an inhibitor of the activity of a molecular variant of a polypeptide of the EDN/EDNR/ECE signaling system comprising the steps of:

(a) contacting the protein, the solid support of the invention or a cell expressing a molecular variant gene comprising a polynucleotide or the gene or the vector of the invention in the presence of components capable of providing a detectable signal in response to drug activity with a compound to be screened for inhibiting activity; and

(b) detecting the presence or absence of a signal or increase or decrease of a signal generated from the inhibiting activity, wherein the absence or decrease of the signal is indicative for a putative inhibitor.

In a preferred embodiment of the method of the invention said cell is a cell, obtained by the method of the invention or comprised in the transgenic non-human animal as described supra.

In a still further embodiment the present invention relates to a method of identifying and obtaining a pro-drug or drug capable of modulating the activity of a molecular variant of a polypeptide of the EDN/EDNR/ECE signaling system or its gene product comprising the steps of:

(a) contacting the host cell, the cell obtained by the method of the invention, the

polypeptide or the solid support of the invention with the first molecule known to be bound by a protein of the EDN/EDNR/ECE signaling cascade to form a first complex of said polypeptide and said first molecule;

(b) contacting said first complex with a compound to be screened, and

(c) measuring whether said compound displaces said first molecule from said first complex.

Advantageously, in said method said measuring step comprises measuring the formation of a second complex of said protein and said inhibitor candidate. Preferably, said measuring step comprises measuring the amount of said first molecule that is not bound to said protein.

In a particularly preferred embodiment of the above-described method of said first molecule is a agonist or antagonist of the EDN polypeptides or the EDNR receptors of the invention or a substrate and/or a inhibitor and/or a modulator of the ECE polypeptides of the invention, e.g., with a radioactive or fluorescent label.

In a still another embodiment the present invention relates to a method of identifying and obtaining an inhibitor capable of modulating the activity of a molecular variant of a polypeptide of the EDN/EDNR/ECE signaling system or its gene product comprising the steps of:

(a) contacting the host cell or the cell obtained by the method of the invention, the protein or the solid support of the invention with the first molecule known to be bound by a protein of the EDN/EDNR/ECE signaling cascade to form a first complex of said protein and said first molecule;

(b) contacting said first complex with a compound to be screened, and

(c) measuring whether said compound displaces said first molecule from said first complex.

In a preferred embodiment of the method of the invention said measuring step comprises measuring the formation of a second complex of said protein and said compound.

In another preferred embodiment of the method of the invention said measuring step comprises measuring the amount of said first molecule that is not bound to said protein.

In a more preferred embodiment of the method of the invention said first molecule is labeled.

The invention furthermore relates to a method for the production of a pharmaceutical composition comprising the steps of the method as described supra; and the further step of formulating the compound identified and obtained or a derivative thereof in a pharmaceutically acceptable form.

The therapeutically useful compounds identified according to the method of the invention may be formulated and administered to a patient as discussed above. For uses and therapeutic doses determined to be appropriate by one skilled in the art and for definitions of the term "pharmaceutical composition" see infra.

Furthermore, the present invention encompasses a method for the preparation of a pharmaceutical composition comprising the steps of the above-described methods; and formulating a drug or pro-drug in the form suitable for therapeutic application and preventing or ameliorating the disorder of the subject diagnosed in the method of the invention.

Drugs or pro-drugs after their *in vivo* administration are metabolized in order to be eliminated either by excretion or by metabolism to one or more active or inactive metabolites (Meyer, J. Pharmacokinet. Biopharm. 24 (1996), 449-459). Thus, rather than using the actual compound or inhibitor identified and obtained in accordance with the methods of the present invention a corresponding formulation as a pro-drug can be used which is converted into its active in the patient. Precautionary measures that may be taken for the application of pro-drugs and drugs are described in the literature; see, for review, Ozama, J. Toxicol. Sci. 21 (1996), 323-329).

In a preferred embodiment of the method of the present invention said drug or prodrug is a derivative of a medicament as defined hereinafter.

The present invention also relates to a method of diagnosing a disorder related to the presence of a molecular variant of a gene of the EDN/EDNR/ECE signaling system or susceptibility to such a disorder comprising determining the presence of a polynucleotide or the gene of the invention in a sample from a subject.

In accordance with this embodiment of the present invention, the method of testing the status of a disorder or susceptibility to such a disorder can be effected by using a polynucleotide gene or nucleic acid of the invention, e.g., in the form of a Southern or Northern blot or *in situ* analysis. Said nucleic acid sequence may hybridize to a coding region of either of the genes or to a non-coding region, e.g. intron. In the case that a complementary sequence is employed in the method of the invention, said nucleic acid molecule can again be used in Northern blots. Additionally, said testing can be done in conjunction with an actual blocking, e.g., of the transcription of the gene and thus is expected to have therapeutic relevance. Furthermore, a primer or oligonucleotide can also be used for hybridizing to one of the above mentioned genes of the EDN/EDNR/ECE signaling system or corresponding mRNAs. The nucleic acids used for hybridization can, of course, be conveniently labeled by incorporating or attaching, e.g., a radioactive or other marker. Such markers are well known in the art. The labeling of said nucleic acid molecules can be effected by conventional methods.
Additionally, the presence or expression of variant genes of the EDN/EDNR/ECE signaling system can be monitored by using a primer pair that specifically hybridizes to either of the corresponding nucleic acid sequences and by carrying out a PCR reaction according to standard procedures. Specific hybridization of the above mentioned probes or primers preferably occurs at stringent hybridization conditions. The term "stringent hybridization conditions" is well known in the art; see, for example, Sambrook et al., "Molecular Cloning, A Laboratory Manual" second ed., CSH Press, Cold Spring Harbor, 1989; "Nucleic Acid Hybridisation, A Practical Approach", Hames and Higgins eds., IRL Press, Oxford, 1985. Furthermore, the mRNA, cRNA, cDNA or genomic DNA obtained from the subject

may be sequenced to identify mutations which may be characteristic fingerprints of mutations in the genes of the EDN/EDNR/ECE signaling system. The present invention further comprises methods wherein such a fingerprint may be generated by RFLPs of DNA or RNA obtained from the subject, optionally the DNA or RNA may be amplified prior to analysis, the methods of which are well known in the art. RNA fingerprints may be performed by, for example, digesting an RNA sample obtained from the subject with a suitable RNA-Enzyme, for example RNase T_1, RNase T_2 or the like or a ribozyme and, for example, electrophoretically separating and detecting the RNA fragments as described above.
Further modifications of the above-mentioned embodiment of the invention can be easily devised by the person skilled in the art, without any undue experimentation from this disclosure; see, e.g., the examples. An additional embodiment of the present invention relates to a method wherein said determination is effected by employing an antibody of the invention or fragment thereof. The antibody used in the method of the invention may be labeled with detectable tags such as a histidine flags or a biotin molecule.

The invention relates to a method of diagnosing a disorder related to the presence of a molecular variant of a gene of the EDN/EDNR/ECE signaling system or susceptibility to such a disorder comprising determining the presence of a polypeptide or the antibody of the invention.

In a preferred embodiment of the above described method said disorder is cancer, a cardiovascular disease or a disorder related to abnormal angiogenesis.

In a preferred embodiment of the present invention, the above described method is comprising PCR, ligase chain reaction, restriction digestion, direct sequencing, nucleic acid amplification techniques, hybridization techniques or immunoassays. Said techniques are very well known in the art.

Moreover, the invention relates to a method of detection of the polynucleotide or the gene of the invention in a sample comprising the steps of
(a) contacting the solid support described supra with the sample under

conditions allowing interaction of the polynucleotide or the gene of the invention with the immobilized targets on a solid support and;

(b) determining the binding of said polynucleotide or said gene to said immobilized targets on a solid support.

The invention also relates to an in vitro method for diagnosing a disease comprising the steps of the method described supra, wherein binding of said polynucleotide or gene to said immobilized targets on said solid support is indicative for the presence or the absence of said disease or a prevalence for said disease.

The invention furthermore relates to a diagnostic composition comprising the polynucleotide, the gene, the vector, the polypeptide or the antibody of the invention.

In addition, the invention relates to a pharmaceutical composition comprising the polynucleotide, the gene, the vector, the polypeptide or the antibody of the invention.

These pharmaceutical compositions comprising, e.g., the antibody may conveniently be administered by any of the routes conventionally used for drug administration, for instance, orally, topically, parenterally or by inhalation. Acceptable salts comprise acetate, methylester, HCl, sulfate, chloride and the like. The compounds may be administered in conventional dosage forms prepared by combining the drugs with standard pharmaceutical carriers according to conventional procedures. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation. It will be appreciated that the form and character of the pharmaceutically acceptable character or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof. The pharmaceutical carrier employed may be, for example, either

a solid or liquid. Exemplary of solid carriers are lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary of liquid carriers are phosphate buffered saline solution, syrup, oil such as peanut oil and olive oil, water, emulsions, various types of wetting agents, sterile solutions and the like. Similarly, the carrier or diluent may include time delay material well known to the art, such as glyceryl mono-stearate or glyceryl distearate alone or with a wax.

The dosage regimen will be determined by the attending physician and other clinical factors; preferably in accordance with any one of the above described methods. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Progress can be monitored by periodic assessment.

Furthermore, the use of pharmaceutical compositions which comprise antisense-oligonucleotides which specifically hybridize to RNA encoding mutated versions of a gene of the EDN/EDNR/ECE signaling system according to the invention or which comprise antibodies specifically recognizing mutated polypeptide of the invention but not or not substantially the functional wild-type form is conceivable in cases in which the concentration of the mutated form in the cells should be reduced.

Thanks to the present invention the particular drug selection, dosage regimen and corresponding patients to be treated can be determined in accordance with the present invention. The dosing recommendations will be indicated in product labeling by allowing the prescriber to anticipate dose adjustments depending on the considered patient group, with information that avoids prescribing the wrong drug to the wrong patients at the wrong dose.

In another embodiment the present invention relates to the use of the polynucleotide, the gene, the vector, the polypeptide, the polynucleotides having at a position corresponding to position 3745/6 of the EDN-1 gene (Accession No: J05008) an insertion of an A or at a position corresponding to position 7267 of the EDN-1 gene (Accession No: J05008) an A, or the antibody of the invention for the

preparation of a diagnostic composition for diagnosing a disease.

By "position 3745/6" it is meant that said polynucleotide comprises an additional nucleotide which is inserted between positions 3745 and position 3746 of the corresponding wild type version of said polynucleotide.

A gene encoding a functional and expressible polypeptide of the invention can be introduced into the cells which in turn produce the protein of interest. Gene therapy, which is based on introducing therapeutic genes into cells by *ex-vivo* or *in-vivo* techniques is one of the most important applications of gene transfer. Suitable vectors and methods for *in-vitro* or *in-vivo* gene therapy are described in the literature and are known to the person skilled in the art; see, e.g., Giordano, Nature Medicine 2 (1996), 534-539; Schaper, Circ. Res. 79 (1996), 911-919; Anderson, Science 256 (1992), 808-813; Isner, Lancet 348 (1996), 370-374; Muhlhauser, Circ. Res. 77 (1995), 1077-1086; Wang, Nature Medicine 2 (1996), 714-716; WO94/29469; WO 97/00957 or Schaper, Current Opinion in Biotechnology 7 (1996), 635-640, and references cited therein. The gene may be designed for direct introduction or for introduction via liposomes, or viral vectors (e.g. adenoviral, retroviral) into the cell. Preferably, said cell is a germ line cell, embryonic cell, or egg cell or derived therefrom, most preferably said cell is a stem cell.

As is evident from the above, it is preferred that in the use of the invention the nucleic acid sequence is operatively linked to regulatory elements allowing for the expression and/or targeting of the polypeptides of the invention to specific cells. Suitable gene delivery systems that can be employed in accordance with the invention may include liposomes, receptor-mediated delivery systems, naked DNA, and viral vectors such as herpes viruses, retroviruses, adenoviruses, and adeno-associated viruses, among others. Delivery of nucleic acids to a specific site in the body for gene therapy may also be accomplished using a biolistic delivery system, such as that described by Williams (Proc. Natl. Acad. Sci. USA 88 (1991), 2726-2729). Standard methods for transfecting cells with recombinant DNA are well known to those skilled in the art of molecular biology, see, e.g., WO 94/29469; see also supra. Gene therapy may be carried out by directly administering the

recombinant DNA molecule or vector of the invention to a patient or by transfecting cells with the polynucleotide or vector of the invention ex vivo and infusing the transfected cells into the patient.

In a further embodiment the present invention relates to the use of the polynucleotide, the gene, the vector, the polypeptide or the antibody of the invention for the preparation of a pharmaceutical composition for treating a disease.

In a more preferred embodiment of the use of the present invention said disease is cancer or a cardiovascular disease comprising coronary heat disease, hypertension, atherosclerosis or a disease related to abnormal angiogenesis.

Finally, the present invention relates to a diagnostic kit for detection of a single nucleotide polymorphism comprising the polynucleotide, the gene, the vector, the polypeptide, the antibody, the host cell, the transgenic non-human animal or the solid support of the invention.

The kit of the invention may contain further ingredients such as selection markers and components for selective media suitable for the generation of transgenic cells and animals. The kit of the invention may advantageously be used for carrying out a method of the invention and could be, inter alia, employed in a variety of applications, e.g., in the diagnostic field or as research tool. The parts of the kit of the invention can be packaged individually in vials or in combination in containers or multicontainer units. Manufacture of the kit follows preferably standard procedures which are known to the person skilled in the art. The kit or diagnostic compositions may be used for methods for detecting expression of a mutant form of the polypeptides, genes or polynucleotides in accordance with any one of the above-described methods of the invention, employing, for example, immunoassay techniques such as radioimmunoassay or enzymeimmunoassay or preferably nucleic acid hybridization and/or amplification techniques such as those described herein before and in the Examples.

The figures illustrate the invention

Figure 1: The figure shows reference sequences for the exons of the EDN-2 gene, including the adjacent intron regions.

Figure 2: The figure shows reference sequences for two exons of the EDNRB gene, including the adjacent intron regions.

The invention will now be described by reference to the following biological Examples which are merely illustrative and are not constructed as a limitation of the scope of the present invention.

Example 1: Isolation of genomic DNA from human blood, generation and purification of endothelin gene fragments

Genomic DNA was obtained by standard ion exchange chromatography techniques (Quiagen kits for isolation of genomic DNA from blood). Blood from all the individuals that were tested (volunteers/patients from the department of Pharmacology at the Charite, Berlin) was obtained under consideration of all legal, ethical, medical and bureaucratical requirement of the Charite Klinikum in Berlin, Germany.

Several sets of novel genomic sequences had to be obtained for the optimization of the PCR fragments of the EDN-2 and the EDNRB gene (Example 2). Then specific oligonucleotide primers, 2 for each fragment, were applied to obtain defined DNA fragments by polymerase chain reaction (PCR) containing specific parts of the genes, which are involved in the regulated function of blood vessels (EDN 1-3, EDNRA, EDNRB and ECE-1 gene). These specific oligonucleotide primers were designed to bind to sequences upstream and downstream of various exons of the genes, which are involved in the regulated function of blood vessels (EDN 1-3, EDNRA, EDNRB and ECE-1 gene). The resulting DNA fragments were to encode not only exon sequences, but also some intron sequences at the exon-intron boundaries. Such intronic sequences adjacent to the exons are known to be important for correct splicing and subsequent expression of the mRNA, which

encodes for the respective protein. Oligonucleotide primer pairs that were optimized for each of the PCR fragments, synthesized and purified by affinity chromatography (OPC cartridges). The primer sequences for the amplification of fragments, which include SNP-positions are listed in Table 1.

Polymerase chain reactions for the fragments, which include SNP positions were performed under conditions, that were optimized for each of these fragments. These fragments cover the respective exons, as well as regulatory regions, like promoter, 5'-UTR and 3'-UTR (see Table 1). PCR reactions were carried out for all these fragments in a reaction volume of 50µl. 100ng DNA template was added to standard PCR-buffer containing 1,5mM MgCl2 (Quiagen, Hilden), 200µM dNTP's (Quiagen, Hilden), 50pMol each primer (Metabion, Munich) and 1 U Taq polymerase (Quiagen, Hilden). All PCR's were performed on a Perkin Elmer thermocycler (model 9700) with an initial denaturation step of 3 min at 94°C and 31 amplification cycles of denaturation 94°C for 30 sec, , primer annealing depending on the primer's melting temperature (PCR conditions: **A-F**) for 30 sec, and 30 sec for 72°C followed by a final extension of 72°C for 5 min. For the single PCR conditions **A-F** the following annealing temperatures were applied: **A**: 57°C; **B**: 58°C; **C**: 56°C; **D**: 61°C; **E**: 59°C; **F**: 63°C.

The optimized PCR-conditions and the resulting size of the desired and obtained fragments are listed in Table 1. The defined DNA fragments containing specific parts of the human genes, which are involved in the regulated function of blood vessels, were processed to remove nonincorporated nucleotides and buffer components that otherwise interfere with the subsequent determination of the individual „endothelin" genotype by direct DNA sequencing. For this purification, standard ion exchange chromatography techniques were used (Quiagen kits for PCR fragment purification). For all of the fragments, sufficient yields of purified fragments, suitable for direct DNA sequence analyses, were obtained.

Example 2: Extended analysis of genomic EDN-2 and EDNRB sequences for appropriate generation of PCR fragments

Because for certain regions of the EDN-2 gene and of the EDNRB gene no sequence information was available in the databases, the generation of appropriate PCR fragments was not possible in these cases. Therefore these missing sequence regions were analysed by sequence analysis. In Table 3 all these new and unpublished sequence regions of the EDN-2, as well as of the EDNRB gene are listed. These sequences are also a part of the reference sequences (see Figure 1 and 2). The knowledge of these novel sequence information was used at Epidauros for the genetic analysis of the EDN-2 and the EDNRB gene. In both genes different new SNP's could be detected (see Example 3 and the description above), 4 of which are localized within the new and unpublished sequence regions (EDN-2 gene: exon 2 (-97, intron 1) and exon 4 (+134, intron 4); EDNRB gene: exon 4 (-122, intron 3) and exon 5 (-69, intron 4); see Table 2 and 3). The deviant bases in the sequences are bold, underlined and in another type size (see Table 3). Thus, the knowledge of the new and unpublished sequence regions on the one hand is absolutely necessary to detect these 4 novel proprietary alleles, on the other hand they have to be needed for the analysis of the EDN-2 and the EDNRB gene.

Example 3: Identification of different „endothelin" gene alleles by sequence determination in various individuals

For sequence analysis of relevant regions of the human genes for EDN-1, EDN-2, EDN-3, EDNRA, EDNRB and ECE-1 from 56 different individuals, PCR amplifiaction of the relevant fragments of these genes were carried out (see Table 1) and the purified PCR products subsequently sequenced with established methods (ABI dyeterminator cycle sequencing). A very important parameter that was needed to consider using this approach was that each normal human individual harbors two copies of the respective genes, which are involved in the

regulated function of blood vessels. Because of this diploidy (of autosomal genes; EDN-1, EDN-2, EDN-3, EDNRA, EDNRB and ECE-1 are autosomal encoded), great care had to be taken in the evaluation of the sequences to be able to identify unambiguously not only homozygous sequence variations but also heterozygous variations. Because of that, it was never relied on only one determined sequence, but always obtained at least two sequences from each defined gene fragment (referring to EDN-1, EDN-2, EDN-3, EDNRA, EDNRB and ECE-1) from each individual, by sequencing both opposite DNA strands.

For the initial evaluation of gene variations in the human population, sequence analyses of the relevant regions of all 6 genes (EDN-1, EDN-2, EDN-3, EDNRA, EDNRB and ECE-1) were carried out from the genomic DNA from 56 different individuals. This number of individual samples was then extended for a screening for specific SNP's in the EDN-1 gene, one of which have been analysed from 1983 individuals. The sequences were inspected for the occurence of DNA sequences that were deviant either from the published sequences of the 6 genes (EDN-1, EDN-2, EDN-3, EDNRA, EDNRB and ECE-1), or deviant from specifically created Epidauros reference sequences (see Figure 1 and 2). These reference sequences are both considered as „wildtype" sequences in all of this work. Because population genetics enables a calculation of the expected frequency of homozygous vs. heterozygous alleles of a defined gene (Hardy-Weinberg distribution, using the formulas $p = (2 \times AA + 1 \times Aa)/2N$ and $p + q = 1$: AA = number of probands homozygous for the wt-allele, Aa = number of heterozygotes, N = size of the sample test, p = frequency of the wt-allele, q = frequency of the mut-allele, q^2 = frequency of the genotype homozygous for the mut-allele), it was possible to confirm the predicted (using these formulas) distribution of homozygous vs. heterozygous alleles and deviations with the experimental findings (see Table 2). This serves as internal control and confirmation that a detected sequence deviation indeed represents a novel allele.

In total 70 polymorphisms could be found in the EDN-1, EDN-2, EDN-3 genes, the two endothelin receptor genes (EDNRA, EDNRB) and in the ECE-1 gene. 55 of all polymorphisms found are new und presently not published in the literature. The following table gives an overview over all SNP's, which described in the literature and newly found in our study:

Gene	Total number of known SNP's	SNP's, which are only described in the literature	SNP's, which are described in the literature and also found at Epidauros	New SNP's found at Epidauros
ECE-1	33	2	1	29
EDN-1	13	5	3	5
EDN-2	10	2	1	7
EDN-3	7	2	1	3
EDNRA	17	7	8	2
EDNRB	31	21	1	9

In regard to the 55 newly found SNP's, the different types of polymorphisms that were detected, as well as their distribution over the 6 genes (EDN-1, EDN-2, EDN-3, EDNRA, EDNRB and ECE-1) and the possible meaning of the new SNP's are described in more detail below. The exact positions and further details of the novel alleles, including the exact novel sequence and sequence deviation, and the homozygous vs. heterozygous distribution of the respective allele in the population are listed in Table 2. The expected frequency for homozygotes of the variant allele were calculated on the basis of the Hardy-Weinberg distribution (formulas see above). The deviant base in the sequence is bold and underlined.

Among the single nucleotide polymorphisms which have been newly identified for ECE-1, twenty single nucleotide polymorphisms reside in intronic sequences, one single nucleotide polymorphism results in an Thr to Ile amino acid exchange at position 324, one single nucleotide polymorphism residing at a wobble position may effect interaction with a tRNA during translation of mRNA encoded by a gene comprising said single nucleotide polymorphisms, one single nucleotide polymorphism resides in the 3'-UTR of ECE-1 and six single nucleotide polymorphisms in its promoter region. It is reasonable that each single nucleotide polymorphism effects either direct function of the ECE-1 polypeptide or its expression or translation. The above described single nucleotide polymorphisms are useful as, e.g. diagnostic markers since they could be correlated with phenotypes resulting thereof, such as coronary heart diseases or hypertension.

The five single nucleotide polymorphisms newly identified for EDN-1 are localized in intronic sequences and in the 3'-UTR. As described for ECE-1 said single nucleotide polymorphisms are useful as, e.g. diagnostic markers since they could be correlated with phenotypes resulting thereof, such as coronary heart diseases.
For EDN-2, seven single nucleotide polymorphisms have been newly identified. Said single nucleotide polymorphisms reside in introns or 5'- or 3'-UTR sequences. As described for ECE-1 said single nucleotide polymorphisms are useful as, e.g. diagnostic markers since they may be correlated with phenotypes resulting thereof, such as coronary heart disease.
For EDN-3, three single nucleotide polymorphisms have been newly identified, for EDNRA two single nucleotide polymorphisms and for EDNRB nine single nucleotide polymorphisms could be newly detected. As described for ECE-1 said single nucleotide polymorphisms are useful as, e.g. diagnostic markers since they may be correlated with phenotypes resulting thereof, such as cardiovascular diseases.

Example 4: Distribution of SNP's in populations that are stratified for cardiovascular diseases and risk factors

To identify potential direct correlations of polymorphisms found in the 6 genes that were analysed (EDN 1-3, EDNRA, EDNRB and ECE-1) with clinical relevant phenotypes in humans, patients from a study at the Institute of Clinical Pharmacology, University Medical Center Charite in Berlin were subjected to the determination of polymorphisms as described in example 3. The patient collective that was analysed comprises of 4 subgroups with defined cardiovascular phenotype: controls (without coronary heart disease), low risk patients (no coronary heart disease, but atherosclerosis; no risk factors), patients with an early onset of the coronary heart disease (age of onset: < 40 years; diverse risk factors) and patients with a severe disease (age of onset: < 50 years; diverse risk factors). For the evaluation, if some of the 55 newly found SNP's are overrepresented and underrepresented in these patient subgroups, respectively the exact allele distribution was determined. The percentages for all novel alleles distributed on the

4 patient groups are listed in the following table.

Gene	SNP	Controls		Low risk		Severe disease		Early manifestation	
		wt allele (%)	***mut allele (%)***	**wt allele (%)**	***mut allele (%)***	**wt allele (%)**	***mut allele (%)***	**wt allele (%)**	***mut allele (%)***
EDN-1	T5783G (intron 2)	0	*100*	28.6	*71.4*	31.3	*68.7*	7.1	*92.9*
	T3980C (intron 1)	100	*0*	92.9	*7.1*	100	*0*	100	*0*
	9174/5insT (intron 4)	83.3	*16.7*	100	*0*	87.5	*12.5*	100	*0*
	T10045C (exon 5, 3'-UTR)	75	*25*	92.9	*7.1*	87.5	*12.5*	100	*0*
	T10092C (exon 5, 3'-UTR)	100	*0*	92.9	*7.1*	100	*0*	100	*0*
EDN-2	T+134C (intron 4)	63.6	*36.4*	85.7	*14.3*	87.5	*12.5*	100	*0*
	A-40/G/G-40A (exon 1, 5'-UTR)	83.3	*16.7*	100	*0*	93.8	*6.2*	92.9	*7.1*
	C-15T (exon 1, 5'-UTR)	100	*0*	85.7	*14.3*	87.5	*12.5*	100	*0*
	C+47A (exon 1, intron 1)	100	*0*	92.9	*7.1*	100	*0*	100	*0*
	G-97A (exon 2, intron 1)	75	*25*	78.6	*21.4*	81.3	*18.7*	35.7	*64.3*
	T-71C (exon 3, intron 2)	100	*0*	92.9	*7.1*	100	*0*	100	*0*
	G+40A (exon 5, 3'-UTR)	100	*0*	92.9	*7.1*	93.7	*6.3*	100	*0*
EDN-3	G40215A (intron 2)	100	*0*	78.6	*21.4*	100	*0*	85.7	*14.3*
	G59430C (intron 2)	91.7	*8.3*	85.7	*14.3*	100	*0*	100	*0*
	C63843T (exon 6, 3'-UTR)	91.7	*8.3*	85.7	*14.3*	100	*0*	100	*0*
EDNRA	1366delA (exon 8, 3'-UTR)					50	*50*	0	*100*
	A1830G (exon 8, 3'-UTR)	100	*0*	100	*0*	100	*0*	92.9	*7.1*
EDNRB	T749G (5'-flanking region)	100	*0*	92.9	*7.1*	93.8	*6.2*	92.9	*7.1*

	G937C (5'-flanking region)	100	0	100	0	93.8	6.2	92.9	7.1
	A1112G (exon 1, 5'-UTR)	66.7	33.3	85.7	14.3	81.3	18.7	100	0
	T-122C (exon 4, intron 3)	91.7	8.3	100	0	81.3	18.7	78.6	21.4
	T-69G (exon 5, intron 4)	100	0	100	0	93.8	6.2	92.9	7.1
	G1048A (exon 7, 3'-UTR)	100	0	100	0	93.8	6.2	92.9	7.1
	G1658C (exon 7, 3'-UTR)	100	0	100	0	100	0	92.9	7.1
	C1912T (exon 7, 3'-UTR)	91.7	8.3	92.9	7.1	93.8	6.2	100	0
	C2130T (exon 7, 3'-UTR)					83.3	16.7	100	0
ECE-1	A9439C (prom A2)	100	0	64.3	35.7	56.3	43.7	78.6	21.4
	G109728A (prom B1)	100	0	61.5	38.5	68.8	31.2	78.6	21.4
	C86513T (intron 6)	100	0	57.1	42.9	62.5	37.5	69.2	30.8
	G75354A (exon 9, Thr/ Ile)	91.7	8.3	85.7	14.3	81.3	18.7	92.3	7.7
	G72974A (intron 10)	100	0	100	0	80	20	100	0
	A61579G (exon 14)	100	0	92.9	7.1	81.3	18.7	85.7	14.3
	C53217T (intron 17)	100	0	100	0	86.7	13.3	92.9	7.1
	G53127A (intron 17)	100	0	100	0	86.7	13.3	100	0
	G85627A (intron 6)	100	0	92.9	7.1	100	0	85.7	14.3
	A85472G (intron 7)	41.7	58.3	50	50	56.3	43.7	42.9	57.1
	C85424T (intron 7)	41.7	58.3	57.1	42.9	56.3	43.7	50	50
	C83924T (intron 8)	100	0	57.1	42.9	62.5	37.5	78.6	21.4
	T83847C (intron 8)	41.7	58.3	50	50	50	50	50	50
	75431delC (intron 8)	100	0	100	0	93.8	6.2	100	0
	C75102T (intron 9)	91.7	8.3	64.3	35.7	68.8	31.2	85.7	14.3
	G75099C (intron 9)	91.7	8.3	85.7	14.3	81.3	18.7	92.9	7.1

	G73100A (intron 9)	41.7	*58.3*	50	*50*	43.8	*56.2*	35.7.	*64.3*
	C65946T (intron 11)	100	*0*	92.9	*7.1*	93.8	*6.2*	100	*0*
	C65875T (intron 11)	100	*0*	92.9	*7.1*	100	*0*	100	*0*
	G61752C (intron 13)	33.3	*66.7*	50	*50*	50	*50*	35.7	*64.3*
	C56072T (intron 14)	91.7	*8.3*	71.4	*28.6*	81.3	*18.7*	92.9	*7.1*
	G55860A (intron 15)	100	*0*	100	*0*	93.8	*6.2*	100	*0*
	G55222T (intron 15)	100	*0*	92.9	*7.1*	81.3	*18.7*	92.9	*7.1*
	G53220A (intron 17)	91.7	*8.3*	100	*0*	100	*0*	100	*0*
	C47813T (exon 19, 3'-UTR)	100	*0*	100	*0*	100	*0*	91.7	*8.3*
	C109249T (prom B2/3)	100	*0*	71.4	*28.6*	62.5	*37.5*	84.6	*15.4*
	A9351G (prom A2)	100	*0*	100	*0*	93.8	*6.2*	100	*0*
	G8938T (prom A1)	55.6	*44.4*	30	*70*	12.5	*87.5*	41.7	*58.3*
	G8625T (prom A1)	71.4	*28.6*	33.3	*66.7*	46.1	*53.9*	66.7	*33.3*

In regard to their under- and overrepresentation in the 4 patient subgroups, respectively nearly all of the 55 new SNP's are of great interest, because they represent genetic variety in humans, which may serve as risk factors and potential targets for diagnosis and therapy of Cardiovascular diseases. Some examples: in contrast to the controls the mutant alleles of two promoter SNP's found in the ECE-1 gene (A9439C (prom A2) and G109728A (prom B1)) are overrepresented in the patient subgroups, which are characterized by the existence of coronary heart disease. Likewise the new intron SNP's T5783G (intron 2) in the EDN-1 gene, as well as C83924T (intron 8) in the ECE-1 gene show allele distributions, which point to a correlation with the existence of coronary heart disease.

Example 5: Statistical analyses of correlations between SNP's and coronary heart disease and/or hypertension

Statistical evaluations were performed group spanning specifically in regard to the existence of coronary heart disease and hypertension. This evaluation results in statistically significant correlations with coronary heart disease and/or with hypertension.

The p-values of the statistical evaluations (Chi^2-Test), which result in genotype/phenotype correlations are:

Gene	SNP	coronary heart disease, p-values	Hypertension, p-values
EDN-1	T5783G (intron 2)	**0.037**	0.59
EDN-2	T+134C (intron 4)	**0.016**	0.16
ECE-1	C86513T (intron 6)	**0.039**	0.64
	C83924T (intron 8)	**0.051**	0.78
	G72974A (intron 10)	0.38	**0.011**
	A61579G (exon 14)	0.17	**0.008**
	G53127A (intron 17)	0.44	**0.05**
	C53217T (intron 17)	0.33	**0.013**

The two promoter polymorphisms in the ECE-1 gene, which are localized in the two different known promoter sequences show statistically significant correlations with both the low risk group and the severe disease group. Likewise, the correlation of these two SNP's specifically with the existence of coronary heart disease is statistically significant. The p-values (Chi^2-test) are:

Gene	SNP	controls vs. low risk, p-values	controls vs. severe disease, p-values	coronary heart disease, p-values
ECE-1	A9439C (prom A2)	**0.030**	**0.013**	**0.016**
	G109728A (prom B1)	**0.030**	**0.054**	**0.030**

Example 6: Extended analysis of two SNP's in the EDN-1 gene in a large patient collective

To further validate the correlations found for the gene variations (see Example 5), an extended patient collective encompassing up to 1983 patients was analysed. For that we used SNP's in the EDN-1 gene (3745/6insA, 5'-UTR; G7267A, exon 3, silent), which have already been published (Tiret et al. 1999, Hypertension 33: 1169-1174; Cambien et al. 1999, Am. J. Hum. Genet. 65: 183-191), although for those up to now no data about correlations with coronary heart disease and/or hypertension exist in the literature. Likewise, the known SNP's are good candidates for an extended analysis.

In regard to their allele distribution in the 56 patient screen, these two SNP's in the EDN-1 gene, indicated a potential meaning as a risk factor and as potential targets for diagnosis and therapy of Cardiovascular disease/Atherosclerosis does exist. The following Table show the percentages of these alleles distributed over the 4 patient groups (totally 56 patients):

Gene	SNP	Controls		Low risk		Severe disease		Early manifestation	
		wt allele (%)	***mut allele (%)***	**wt allele (%)**	***mut allele (%)***	**wt allele (%)**	***mut allele (%)***	**wt allele (%)**	***mut allele (%)***
EDN-1	3745/6insA (exon1)	45.5	*54.5*	53.9	*46.1*	31.3	*68.7*	35.7	*64.3*
	G7267A (exon 3, silent)	75	*25*	78.6	*21.4*	62.5	*37.5*	92.9	*7.1*

The statistical evaluations were performed group spanning specifically in regard to the existence of coronary heart disease. The p-values of the statistical evaluations (Chi^2-Test), which result in genotype/phenotype correlations are:

Gene	SNP	coronary heart disease, p-values
EDN-1	3745/6insA (exon 1)	**0.012**
	G7267A (exon 3, silent)	**0.054**

These two SNP's (3745/6insA, 5'-UTR and G7267A, exon 3, silent), both of which are described in the literature (Tiret et al. 1999, Hypertension 33: 1169-1174; Cambien et al. 1999, Am. J. Hum. Genet. 65: 183-191) and also found in the screen described in the above Examples show statistically significant correlations with coronary heart disease. These statistical evaluations refer to large patient collectives (3745/6insA: 1983 patients and G7267A: 1944 patients).

Although these SNP's are published in the literature, this genotype/phenotype correlation is new and unpublished.

Table 1: Primers for the amplification of fragments of the EDN-1, EDN-2, EDN-3, EDNRA, EDNRB and ECE-1 gene

PCR fragment name	PCR primer position	Primer sequence		PCR conditions	fragment size
EDN1 gene:	**Accession J05008**				
exon 1	3763-3782	EnI-Exon 1Bf:	5'-GAT CGC TTT GAG ATC TGA GG-3'	A	298 bp
	4041-4060	EnI-Exon 1Br:	5'-TTG CTC TCT AAG CTG CAG TC-3'		
exon 2	5522-5540	EnI-Exon 2f:	5'-ATC CAG CAT GTC TCT CGG C-3'	A	333 bp
	5836-5854	EnI-Exon 2r:	5'-GGA CTA CCT TCA GAG TCC C-3'		
exon 5	9065-9085	EnI-Exon 5Af neu:	5'-GGT TTT ATC AAA GAG TTG CGG-3'	C	523 bp
	9567-9587	EnI-Exon 5Ar neu:	5'-AAT GTG ACT GTG TGT TTC TGC-3'		
exon 5	9879-9898	EnI-Exon 5Cf:	5'-GAA TAT GAG TCT ACC TCA CC-3'	A	466 bp
	10325-10344	EnI-Exon 5Cr:	5'-CAT AGA CCA ATA TGG CCT GC-3'		
EDN2 gene: see specific reference sequence Figure 1					
exon 1, 5'-UTR	(4) - (23)	EnII-Exon 1f:	5'-GCT TAA TAG CAG GAC GCT GG-3'	D	224 bp
	(+77) - (+94)	EnII-Exon 1r:	5'-CCA GCT GGC TTG GGA GGC-3'		
exon 2	(-125) - (-107)	EnII-Exon 2f:	5'-CTT GAG CAG CAA GAG TGC C-3'	B	369 bp
	(+67) - (+86)	EnII-Exon 2r:	5'-GTC AAC GTT CCC TAG CAT GC-3'		
exon 3	(-159) - (-142)	EnII-Exon 3f:	5'-GGC ACC CTG CTG GCA TGG-3'	B	387 bp
	(+86) - (+104)	EnII-Exon 3r:	5'-CAA CTT GCC AGT TCG CTC C-3'		
exon 4	(-131) - (-113)	EnII-Exon 4f:	5'-GGG GTG GAA ATG GAT CTG C-3'	B	436 bp
	(+186) - (+205)	EnII-Exon 4r:	5'-AGA GCA CAG TGG GTA AGA GC-3'		
exon 5	(-80) - (-61)	EnII-Exon 5Af:	5'-CAT TGC CCC TGC TCT CAT GG-3'	B	499 bp
	914 - 933	EnII-Exon 5Ar:	5'-CAG ACA GGA CAC TCC TCA GG-3'		

EDN3 gene: Accession AL035250					
exon 2	39755-39775	EnIII-Exon 2f:	5'-GCA GAG CTT TGG AAA CTT TGC-3'	**E**	546 bp
	40282-40300	EnIII-Exon 2r:	5'-GGC TCT GGG CTA ACT GAG C-3'		
exon 3	59327-59346	EnIII-Exon 3f:	5'-CCA GAA CCT TCC TAA GAC CC-3'	**B**	460 bp
	59768-59786	EnIII-Exon 3r:	5'-AGG ACG ACA GTA GGT CAG G-3'		
exon 6	63480-63498	EnIII-Exon 6Cf:	5'-GCT GTG CTT GAT ACC CAC C-3'	**B**	526 bp
	63986-64005	EnIII-Exon 6Cr:	5'-CCC ACT CAA ATG CCG TTT CC-3'		
EDNRA gene:	**Accession D11151**				
exon 8, 3'-UTR	1082-1101	EnA-Exon 8Df:	5'-CTG AGA CTT TCA GTG CAC TG-3'	**C**	419 bp
	1481-1500	EnA-Exon 8Dr:	5'-CAC AGT CTG TAA ACA TCT GG-3'		
exon 8, 3'-UTR	1394-1413	EnA-Exon 8Ef:	5'-GTA TGA ACC TAA CTC CCC AC-3'	**A**	519 bp
	1893-1912	EnA-Exon 8Er:	5'-CAC AGG AAA CAA TAT GAC CC-3'		
EDNRB gene:	**Accession D13162**				
5'-flanking region	474-493	EnB-Prom Bf:	5'-ATT GAA CCT TAT TCT GGG GC-3'	**B**	559 bp
	1012-1030	EnB-Prom Br:	5'-TTC CTG ATG CCC TCT CAG C-3'		
exon 1, 5'-UTR	939-956	EnB-Exon 1Af:	5'-CAC ACC CCT TCC AGA ACG-3'	**A**	549 bp
	1469-1487	EnB-Exon 1Ar:	5'-CCT GTC TCC TTT AGG CAC C-3'		
EDNRB gene: see specific reference sequence Figure 2					
exon 4	(-152) – (-133)	EnB-Exon 4f:	5'-TGT TCA GTA AGT GTG GCC TG-3'	**D**	431 bp
	(+88) – (+109)	EnB-Exon 4r:	5'-AGA AAA AGG AAA TAT GCT CTG G-3'		
EDNRB gene: see specific reference sequence Figure 2					
exon 5	(-128) – (-109)	EnB-Exon 5f:	5'-CCA TAT AAA GCT CAG TGT CC-3'	**D**	327 bp
	(+29) – (+47)	EnB-Exon 5r:	5'-GGA AAC ACT TCT GAG TGG C-3'		
	Accession D13168				

exon 7, 3'-UTR	696-716	EnB-Exon 7Cf:	5'-ACA TTT AAA TGA TCA GGA GGG-3'	A	565 bp
	1241-1260	EnB-Exon 7Cr:	5'-CCA TTT TAA CCA CAG CAT GG-3'		
exon 7, 3'-UTR	1620-1638	EnB-Exon 7Ef:	5'-CCT AAC GTT CGT CAT TGC C-3'	A	521 bp
	2121-2140	EnB-Exon 7Er:	5'-GAG AAG GAA AGG GTA TCA GG-3'		
exon 7, 3'-UTR	1965-1984	EnB-Exon 7Ff:	5'-CAA TCA CTT TTT CAG AGG CC-3'	A	570 bp
	2515-2534	EnB-Exon 7Fr:	5'-TCC TTT GGC CAT ATG TAA GC-3'		
ECE-1 gene	**Accession AL031005**				
exon 6	86949-86968	EcoE Ex6f:	5'-CTT TCC TTT GCT CTT GAG GC-3'	C	499 bp
	86470-86489	EcoE Ex6r:	5'-CCA AAG TTT CAC CTG TAT GC-3'		
exon 7	85747-85765	EcoE Ex7f:	5'-GCA GGA ATG TGT CAT CCC G-3'	C	488 bp
	85278-85296	EcoE Ex7r:	5'-GCC TCT CTC TAG AAC CAG G-3'		
exon 8	84201-84218	EcoE Ex8f:	5'-GGA CTT GGC TGG CCA AGC-3'	C	414 bp
	83805-83824	EcoE Ex8r:	5'-GGC ATA ACA ACC ATC CTT CC-3'		
exon 9	75505-75523	EcoE Ex9f:	5'-CAT CTC TGA CAA AAG CCC G-3'	C	524 bp
	75000-75019	EcoE Ex9r:	5'-GTT ACG AAA TTT GCC CAA GG-3'		
exon 10	73223-73241	EcoE Ex10f:	5'-AGC AGT AAC CCA CCA AGC C-3'	C	391 bp
	72851-72879	EcoE Ex10r:	5'-GCA TAA GCA CTC CTT CCG G-3'		
exon 11	66323-66342	EcoE Ex11f:	5'-CTT CCA GAA CAA AGC ATG GG-3'	B	584 bp
	65760-65779	EcoE Ex11r:	5'-CTA GAA CAA ACC TGT CAC CC-3'		
ECE-1 gene	**Accession AL031005**				
exon 14	61821-61839	EcoE Ex14f:	5'-GTG TCA TTC CAT CTC GTG C-3'	C	437 bp
	61403-61421	EcoE Ex14r:	5'-TGG GAT AAC AAG GGC ATC C-3'		
exon 15	56182-56201	EcoE Ex15f:	5'-CAG TCC TTC TGT AGA TGT CC-3'	C	462 bp
	55740-55759	EcoE Ex15r:	5'-CTT CCA GAT CTC ACT GGA GG-3'		
exon 16	55342-55360	EcoE Ex16f:	5'-CAT GAC CAC ACC CTT ATC C-3'	A	366 bp
	54995-55014	EcoE Ex16r:	5'-CAT AAT CCA TCA TGC ACA GC-3'		
exon 17	53507-53525	EcoE Ex17f:	5'-GGA TGA ACC GCT TCT CAC C-3'	C	422 bp
	53104-53122	EcoE Ex17r:	5'-CCC AAT TTC GCA GAA GAG G-3'		
exon 19A	48185-48203	EcoE Ex19Af:	5'-CCC TGC TGT TAT AAT GGG G-3'	C	563 bp

	47641-47660	EcoE Ex19Ar:	5'-GTA TTT GTG GCG TAT CTG GC-3'		
	Accession AL031728				
Promoter A fragment 1	9159-9177	EcoE PromA1f:	5'-CCT GCA GCC ATC CAA ATG C-3'	**F**	572 bp
	8606-8615	EcoE PromA1r:	5'-GCC CCA GAC GCC TGG TCC-3'		
Promoter A fragment 2	9609-9627	EcoE PromA2f:	5'-CTG TCC CTG TGA CGT CAG G-3'	**E**	551 bp
	9077-9096	EcoE PromA2r:	5'-GGC CTT TAA GCC AGA GTT CG-3'		
	Accession AL031005				
Promoter B fragment 1	110182-110201	EcoE Prom1f:	5'-AAC TGA CCC ACT TCA GAT CC-3'	**B**	573 bp
	109629-109648	EcoE Prom1r:	5'-TTG GGG TTC TCC TCT GTT CC-3'		
Promoter B fragment 2	109702-109721	EcoE Prom2f:	5'-ACT GAA CAC GTT ACC CCA CC-3'	**B**	525 bp
	109207-109226	EcoE Prom2r:	5'-GAA GCA CAG ACA TCC TTA GC-3'		

Conditions for the different PCR fragments:

PCRs were carried out for all exons in a reaction volume of 50µl. 100ng DNA template was added to standard PCR buffer containing 1,5mM MgCl2 (Quiagen, Hilden), 200µM dNTP's (Quiagen, Hilden), 50 pMol each primer (Metabion, Munich) and 1 U Taq polymerase (Quiagen, Hilden). All PCRs were performed on a Perkin Elmer thermocycler (model 9700) with an initial denaturation step of 3 min at 94°C and 31 amplification cycles of denaturation 94°C for 30 sec, primer annealing depending on the primer´s melting temperature (PCR conditions: A-H) for 30 sec, and 30 sec for 72°C followed by a final extension of 72°C for 5 min. For the single PCR conditions A-H the following annealing temperatures were applied: A: 57°C; B: 58°C; C: 56°C; D: 61°C; E: 59°C; F: 63°C

Table 1: In this table all primer sequences are listed, which are used to amplify the regions of the 6 different genes (EDN1, EDN-2, EDN-3, EDNRA, EDNRB and ECE-1), in which SNP's could be found.

Table 2: New SNP's in genes for EDN-1, EDN-2, EDN-3, EDNRA, EDNRB and ECE-1

PCR fragment name	Position of the variation	frequency: Hetero-zygotes	frequency: Homo-zygotes mutant	frequency: Homo-zygotes mutant (expec-ted, Hardy-Wein-berg)	wt-sequence	wt/mut- and/or mut-sequence
EDN1 gene: Accession J05008						
exon 1	3980	1.8 %	< 1 %	0.01%	f: GAATTACAAGTTAGTGTGTTC r: GAACACACTAACTTGTAATTC	wt/mut: f: GAATTACAAGT/CTAGTGTGTTC r: GAACACACTAA/GCTTGTAATTC mut/mut: f: GAATTACAAGCTAGTGTGTTC r: GAACACACTAGCTTGTAATTC
exon 2	5783	58.9 %	25%	29%	f: TGTAACCCTATTCATTCATTA r: TAATGAATGAATAGGGTTACA	wt/mut: f: TGTAACCCTAT/GTCATTCATTA r: TAATGAATGAA/CTAGGGTTACA mut/mut: f: TGTAACCCTAGTCATTCATTA r: TAATGAATGACTAGGGTTACA
exon 5 A	9174	7.2 %	< 1%	0.2%	f: GTGATTTTTTTAAAATAACAT	wt/mut: f: GTGATTTTTTT/+TAAAATAACAT

					r: ATGTTATTTTAAAAAAATCAC	r: ATGTTATTTTA/+AAAAAAATCAC **mut/mut:** f: GTGATTTTTTTTAAAATAACAT r: ATGTTATTTTAAAAAAAATCAC **wt/mut:**
exon 5 C	10045	11%	< 1%	0.3 %	f: AGATATAATATTTTCATGGTA r: TACCATGAAAATATTATATCT	f: AGATATAATAT/CTTTCATGGTA r: TACCATGAAAA/GTATTATATCT **mut/mut:** f: AGATATAATACTTTCATGGTA r: TACCATGAAAGTATTATATCT
exon 5 C	10092	1.8 %	< 1 %	0.01 %	f: AAAAAGATCATTAAATCAGGA r: TCCTGATTTAATGATCTTTTT	**wt/mut:** f: AAAAAGATCAT/CTAAATCAGGA r: TCCTGATTTAA/GTGATCTTTTT **mut/mut:** f: AAAAAGATCACTAAATCAGGA r: TCCTGATTTAGTGATCTTTTT

EDN2 gene: see specific reference sequence Figure 1

exon 1, 5'-UTR (-15), referring to the ATG startcodon with the first base set to 1

	7.2 %	< 1 %	0.2 %	f: CCAGCCTGCGCGCTCCACCGC r: GCGGTGGAGCGCGCAGGCTGG	**wt/mut:** f: CCAGCCTGCGC/TGCTCCACCGC r: GCGGTGGAGCG/ACGCAGGCTGG **mut/mut:** f: CCAGCCTGCGTGCTCCACCGC r: GCGGTGGAGCACGCAGGCTGG

exon 1, 5'-UTR (-40), referring to the ATG start codon with the first base set to 1

	7.2 %	< 1 %	0.2 %	f: GGCAACAGGCACTCCCTGCTC	**wt/mut:** f: GGCAACAGGCA/GCTCCCTGCTC

			r: GAGCAGGGAGTGCCTGTTGCC	r: GAGCAGGGAGT/CGCCTGTTGCC **mut/mut:** f: GGCAACAGGCGCTCCCTGCTC r: GAGCAGGGAGCGCCTGTTGCC

exon 1, 5'-UTR (+47), referring to the ATG startcodon with the first base set to 1

1.8 %	< 1 %	0.01 %	f: CCTGGGTCTGCCTATCTCTGT r: ACAGAGATAGGCAGACCCAGG	**wt/mut:** f: CCTGGGTCTGC/ACTATCTCTGT r: ACAGAGATAGG/TCAGACCCAGG **mut/mut:** f: CCTGGGTCTGACTATCTCTGT r: ACAGAGATAGTCAGACCCAGG

EDN2 gene: see specific reference sequence Figure 1

exon 2 (-97, intron 1), referring to the region upstream of exon 2

26.8 %	5.4 %	3.6 %	f: CAACTCCCTCGATTATGCAAG r: CTTGCATAATCGAGGGAGTTG	**wt/mut:** f: CAACTCCCTCG/AATTATGCAAG r: CTTGCATAATC/TGAGGGAGTTG **mut/mut:** f: CAACTCCCTCAATTATGCAAG r: CTTGCATAATTGAGGGAGTTG

exon 3 (-71, intron 2), referring to the region upstream of exon 3

1.8 %	< 1 %	0.01 %	f: ATCCTCTCTCTCTTTATTTTT r: AAAAATAAAGAGAGAGAGGAT	**wt/mut:** f: ATCCTCTCTCT/CCTTTATTTTT r: AAAAATAAAGA/GGAGAGAGGAT **mut/mut:** f: ATCCTCTCTCCCTTTATTTTT r: AAAAATAAAGGGAGAGAGGAT

exon 4 (+134, intron 4), referring to the region downstream of exon 4

		10.7 %	3.6 %	0.9 %	f: GGAAAGCCACTTGCCCAAAGT r: ACTTTGGGCAAGTGGCTTTCC	**wt/mut:** f: GGAAAGCCACT/CTGCCCAAAGT r: ACTTTGGGCAA/GGTGGCTTTCC **mut/mut:** f: GGAAAGCCACCTGCCCAAAGT r: ACTTTGGGCAGGTGGCTTTCC
exon 5 A (+40, 3'-UTR), referring to the region downstream of the stopcodon UAG						
		3.9 %	< 1 %	0.04 %	f: AAGCCCGCGGGGAGAGAGGAG r: CTCCTCTCTCCCCGCGGGCTT	**wt/mut:** f: AAGCCCGCGGG/AGAGAGAGGAG r: CTCCTCTCTCC/TCCGCGGGCTT **mut/mut:** f: AAGCCCGCGGAGAGAGAGGAG r: CTCCTCTCTCTCCGCGGGCTT
EDN3 gene: Accession AL035250						
exon 2	40215 (intron 2)	7.2 %	< 1 %	0.2 %	f: TTTGTGGTGAGGAACGTGGCT r: AGCCACGTTCCTCACCACAAA	**wt/mut:** f: TTTGTGGTGAG/AGAACGTGGCT r: AGCCACGTTCC/TTCACCACAAA **mut/mut:** f: TTTGTGGTGAAGAACGTGGCT r: AGCCACGTTCTTCACCACAAA
exon 3	59430 (intron 2)	5.4 %	< 1 %	0.1 %	f: ACCCTTGGGGGCCCCTGAGCA r: TGCTCAGGGGCCCCCAAGGGT	**wt/mut:** f: ACCCTTGGGGG/CCCCCTGAGCA r: TGCTCAGGGGC/GCCCCAAGGGT **mut/mut:** f: ACCCTTGGGGCCCCCTGAGCA r: TGCTCAGGGGCCCCAAGGGT
exon 6 C	63843	5.5 %	< 1 %	0.1 %	f: CAACTTATACCGTCTGACAGT	**wt/mut:** f: CAACTTATACC/TGTCTGACAGT

					r: ACTGTCAGACGGTATAAGTTG	r: ACTGTCAGACG/AGTATAAGTTG **mut/mut:** f: CAACTTATACTGTCTGACAGT r: ACTGTCAGACAGTATAAGTTG
EDNRA gene: Accession D11151						
exon 8, 3'-UTR	1366	42.8 %	28.6 %	25%	f: CCAGTAACTTAACGATTCTTC r: GAAGAATCGTTAAGTTACTGG	**wt/mut:** f: CCAGTAACTTA/del AACGATTCTTC r: GAAGAATCGTT/del TAAGTTACTGG **mut/mut:** f: CCAGTAACTTdelAACGATTCTTC r: GAAGAATCGTdelTAAGTTACTGG
exon 8, 3'-UTR	1830	1.9 %	< 1 %	0.01 %	f: GTGAAAGCAGATGAGCTGTGG r: CCACAGCTCATCTGCTTTCAC	**wt/mut:** f: GTGAAAGCAGA/GTGAGCTGTGG r: CCACAGCTCAT/CCTGCTTTCAC **mut/mut:** f: GTGAAAGCAGGTGAGCTGTGG r: CCACAGCTCACCTGCTTTCAC
EDNRB gene: Accession D13162						
5' flanking region	749	5.5 %	< 1 %	0.1 %	f: GGGAGGAGTCTTTCGAGTTCA r: TGAACTCGAAAGACTCCTCCC	**wt/mut:** f: GGGAGGAGTCT/GTTCGAGTTCA r: TGAACTCGAAA/CGACTCCTCCC **mut/mut:** f: GGGAGGAGTCGTTCGAGTTCA r: TGAACTCGAACGACTCCTCCC
5' flanking region	937	3.8 %	< 1 %	0.04 %	f: TTCCCTCCCTGGCACACCCCT r: AGGGGTGTGCCAGGGAGGGAA	**wt/mut:** f: TTCCCTCCCTG/CGCACACCCCT r: AGGGGTGTGCC/GAGGGAGGGAA

						mut/mut: f: TTCCCTCCCTCGCACACCCCT r: AGGGGTGTGCGAGGGAGGGAA
exon 1, 5'-UTR	1112	15.7 %	2%	1%	f: GAGCGTGGATACTGGCGAAGA r: TCTTCGCCAGTATCCACGCTC	**wt/mut:** f: GAGCGTGGATA/GCTGGCGAAGA r: TCTTCGCCAGT/CATCCACGCTC **mut/mut:** f: GAGCGTGGATGCTGGCGAAGA r: TCTTCGCCAGCATCCACGCTC

see specific reference sequence Figure 2

exon 4 (-122, intron 3), referring to the region upstream of exon 4)

					wt/mut:
12.5 %	< 1 %	0.4 %	f: GTATGATATATACAAACTGGA r: TCCAGTTTGTATATATCATAC	f: GTATGATATAT/CACAAACTGGA r: TCCAGTTTGTA/GTATATCATAC **mut/mut:** f: GTATGATATACACAAACTGGA r: TCCAGTTTGTGTATATCATAC	

EDNRB gene: see specific reference sequence Figure 2

exon 5 (-69, intron 4), referring to the region upstream of exon 5

					wt/mut:
3.6 %	< 1 %	0.04 %	f: TTAAATGTCGTTTTAGAAGAT r: ATCTTCTAAAACGACATTTAA	f: TTAAATGTCGT/GTTTAGAAGAT r: ATCTTCTAAAA/CCGACATTTAA **mut/mut:** f: TTAAATGTCGGTTTAGAAGAT	

						r: ATCTTCTAAACCGACATTTAA
	Accession D13168					
exon 7 C, 3'-UTR	1048	3.8 %	< 1 %	0.04 %	f: TTTCACTATCGTAGCTTAAAC r: GTTTAAGCTACGATAGTGAAA	**wt/mut:** f: TTTCACTATCG/ATAGCTTAAAC r: GTTTAAGCTAC/TGATAGTGAAA **mut/mut:** f: TTTCACTATCATAGCTTAAAC r: GTTTAAGCTATGATAGTGAAA
exon 7 E, 3'-UTR	1658	2%	< 1 %	0.01 %	f: CAATGCAAAAGGTCCTGATTT r: AAATCAGGACCTTTTGCATTG	**wt/mut:** f: CAATGCAAAAG/CGTCCTGATTT r: AAATCAGGACC/GTTTTGCATTG **mut/mut:** f: CAATGCAAAACGTCCTGATTT r: AAATCAGGACGTTTTGCATTG
exon 7 E, 3'-UTR	1912	6.1 %	< 1 %	0.1 %	f: AATTCACACACCATATGGATT r: AATCCATATGGTGTGTGAATT	**wt/mut:** f: AATTCACACAC/TCATATGGATT r: AATCCATATGG/ATGTGTGAATT **mut/mut:** f: AATTCACACATCATATGGATT r: AATCCATATGATGTGTGAATT
exon 7 F, 3'-UTR	2130	12.5 %	< 1 %	0.4 %	f: TCCTGATACCCTTTCCTTCTC r: GAGAAGGAAAGGGTATCAGGA	**wt/mut:** f: TCCTGATACCC/TTTTCCTTCTC r: GAGAAGGAAAG/AGGTATCAGGA **mut/mut:** f: TCCTGATACCTTTTCCTTCTC r: GAGAAGGAAAAGGTATCAGGA

ECE1 gene: Accession AL031005

exon 6	86513 (intron 6)	21.8 %	7.3 %	3.2 %	f: GCAAGGCCAGCAGCCTGCAAA r: TTTGCAGGCTGCTGGCCTTGC	**wt/mut:** f: GCAAGGCCAGC/TAGCCTGCAAA r: TTTGCAGGCTG/ACTGGCCTTGC **mut/mut:** f: GCAAGGCCAGTAGCCTGCAAA r: TTTGCAGGCTACTGGCCTTGC
exon 7	85627 (intron 6)	5.9 %	< 1 %	0.1 %	f: GTTGCAATGCGTGTATCCACT r: AGTGGATACACGCATTGCAAC	**wt/mut:** f: GTTGCAATGCG/ATGTATCCACT r: AGTGGATACAC/TGCATTGCAAC **mut/mut:** f: GTTGCAATGCATGTATCCACT r: AGTGGATACATGCATTGCAAC
exon 7	85424 (intron 7)	45.5 %	3.6 %	6.8 %	f: CCTGGGCTCCCCTAGCTTCAA r: TTGAAGCTAGGGGAGCCCAGG	**wt/mut:** f: CCTGGGCTCCC/TCTAGCTTCAA r: TTGAAGCTAGG/AGGAGCCCAGG

						mut/mut: f: CCTGGGCTCCTCTAGCTTCAA r: TTGAAGCTAGAGGAGCCCAGG
exon 7	85472 (intron 7)	49.1 %	3.6 %	7.8 %	f: CGGAGCAGGAAGGACCGTGCT r: AGCACGGTCCTTCCTGCTCCG	**wt/mut:** f: CGGAGCAGGAA/GGGACCGTGCT r: AGCACGGTCCT/CTCCTGCTCCG **mut/mut:** f: CGGAGCAGGAGGGACCGTGCT r: AGCACGGTCCCTCCTGCTCCG
exon 8	83847 (intron 8)	49.1 %	3.6 %	7.8 %	f: TTTCAAGGACTAGATGAAATG r: GATTTCATCTAGTCCTTGAAA	**wt/mut:** f: TTTCAAGGACT/CAGATGAAATG r: GATTTCATCTA/GGTCCTTGAAA **mut/mut:** f: TTTCAAGGACCAGATGAAATG r: GATTTCATCTGGTCCTTGAAA
ECE1 gene: Accession AL031005						
exon 8	83924 (intron 8)	21.8 %	5.5 %	2.6 %	f: CCCTAGCAGCCGGCGCTCAAG r: CTTGAGCGCCGGCTGCTAGGG	**wt/mut:** f: CCCTAGCAGCC/TGGCGCTCAAG r: CTTGAGCGCCG/AGCTGCTAGGG **mut/mut:** f: CCCTAGCAGCTGGCGCTCAAG r: CTTGAGCGCCAGCTGCTAGGG
exon 9	75099 (intron 9)	12.7 %	< 1 %	0.4 %	f: TCTCGTAAAAGCCCGCCCAGG r: CCTGGGCGGGCTTTTACGAGA	**wt/mut:** f: TCTCGTAAAAG/CCCCGCCCAGG r: CCTGGGCGGGC/GTTTTACGAGA **mut/mut:** f: TCTCGTAAAACCCCGCCCAGG r: CCTGGGCGGGGTTTTACGAGA **wt/mut:**

exon 9	75102 (intron 9)	21.8 %	1.8 %	1.6 %	f: CGTAAAAGCCCGCCCAGGTCC r: GGACCTGGGCGGGCTTTTACG	f: CGTAAAAGCCC/TGCCCAGGTCC r: GGACCTGGGCG/AGGCTTTTACG **mut/mut:** f: CGTAAAAGCCTGCCCAGGTCC r: GGACCTGGGCAGGCTTTTACG
exon 9	75354 (exon 9, Thr/Ile)	12.7 %	< 1 %	0.4 %	f: GGGTGCCAAGGTCTGCAAGGG r: CCCTTGCAGACCTTGGCACCC	**wt/mut:** f: GGGTGCCAAGG/ATCTGCAAGGG r: CCCTTGCAGAC/TCTTGGCACCC **mut/mut:** f: GGGTGCCAAGATCTGCAAGGG r: CCCTTGCAGATCTTGGCACCC
exon 9	75431 (intron 8)	1.8 %	< 1 %	0.01 %	f: CGGGCTTCTACAGGAAGCAAA r: TTTGCTTCCTGTAGAAGCCCG	**wt/mut:** f:CGGGCTTCTAC/delCAGGAAGCAAA r: TTTGCTTCCTG/delGTAGAAGCCCG **mut/mut:** f: CGGGCTTCTAdelCAGGAAGCAAA r: TTTGCTTCCTdelGTAGAAGCCCG

ECE1 gene: Accession AL031005

exon 10	73100 (intron 9)	52.7 %	5.5 %	10.1 %	f: AGCAGGCTGCGGGGAGAGGAG r: CTCCTCTCCCCGCAGCCTGCT	**wt/mut:** f: AGCAGGCTGCG/AGGGAGAGGAG r: CTCCTCTCCCC/TGCAGCCTGCT **mut/mut:** f: AGCAGGCTGCAGGGAGAGGAG r: CTCCTCTCCCTGCAGCCTGCT
exon 10	72974 (intron	4.3 %	< 1 %	0.05 %	f: CGCAGTAGCAGGCTCACCTTC	**wt/mut:** f: CGCAGTAGCAG/AGCTCACCTTC

	10)				r: GAAGGTGAGCCTGCTACTGCG	r: GAAGGTGAGCC/TTGCTACTGCG **mut/mut:** f: CGCAGTAGCAAGCTCACCTTC r: GAAGGTGAGCTTGCTACTGCG **wt/mut:**
exon 11	65946 (intron 11)	3.6 %	< 1 %	0.03 %	f: GAGCTGCCCTCGTGAGACTCC r: GGAGTCTCACGAGGGCAGCTC	f: GAGCTGCCCTC//TGTGAGACTCC r: GGAGTCTCACG/AAGGGCAGCTC **mut/mut:** f: GAGCTGCCCTTGTGAGACTCC r: GGAGTCTCACAAGGGCAGCTC **wt/mut:**
exon 11	65875 (intron 11)	1.8 %	< 1 %	0.01 %	f: ATGGCTGTCACAGCCTTGCAG r: CTGCAAGGCTGTGACAGCCAT	f: ATGGCTGTCAC/TAGCCTTGCAG r: CTGCAAGGCTG/ATGACAGCCAT **mut/mut:** f: ATGGCTGTCATAGCCTTGCAG r: CTGCAAGGCTATGACAGCCAT **wt/mut:**
exon 14	61579 (exon 14)	10.7 %	< 1 %	0.3 %	f: TCCTGAGCTGATCGGCAGTGA r: TCACTGCCGATCAGCTCAGGA	f: TCCTGAGCTGA/GTCGGCAGTGA r: TCACTGCCGAT/CCAGCTCAGGA **mut/mut:** f: TCCTGAGCTGGTCGGCAGTGA r: TCACTGCCGACCAGCTCAGGA
ECE1 gene: Accession AL031005						
						wt/mut:
exon 14	61752 (intron 13)	51%	11.8 %	13.9 %	f: AGATTAATCTGCAGGCTGGAG r: CTCCAGCCTGCAGATTAATCT	f: AGATTAATCTG/CCAGGCTGGAG r: CTCCAGCCTGC/GAGATTAATCT

						mut/mut: f: AGATTAATCTCCAGGCTGGAG r: CTCCAGCCTGGAGATTAATCT **wt/mut:**
exon 15	56072 (intron 14)	16.1 %	< 1 %	0.6 %	f: CAGCACAGGGCGGGAGGCAGG r: CCTGCCTCCCGCCCTGTGCTG	f: CAGCACAGGGC/TGGGAGGCAGG r: CCTGCCTCCCG/ACCCTGTGCTG **mut/mut:** f: CAGCACAGGGTGGGAGGCAGG r: CCTGCCTCCCACCCTGTGCTG
exon 15	55860 (intron 15)	1.8 %	< 1 %	0.01 %	f: GGCTTAGGTCGTATGGGCCCC r: GGGGCCCATACGACCTAAGCC	**wt/mut:** f: GGCTTAGGTCG/ATATGGGCCCC r: GGGGCCCATAC/TGACCTAAGCC **mut/mut:** f: GGCTTAGGTCATATGGGCCCC r: GGGGCCCATATGACCTAAGCC
exon 16	55222 (intron 15)	8.9 %	< 1 %	0.2 %	f: TAAGGCCCTGGAGGGAAAGAC r: GTCTTTCCCTCCAGGGCCTTA	**wt/mut:** f: TAAGGCCCTGG/TAGGGAAAGAC r: GTCTTTCCCTC/ACAGGGCCTTA **mut/mut:** f: TAAGGCCCTGTAGGGAAAGAC r: GTCTTTCCCTACAGGGCCTTA **wt/mut:**

exon 17	53127 (intron 17)	3.6 %	< 1 %	0.04 %	f: AAGAGGAAACGGAAGCTCGCA r: TGCGAGCTTCCGTTTCCTCTT	f: AAGAGGAAACG/AGAAGCTCGCA r: TGCGAGCTTCC/TGTTTCCTCTT **mut/mut:** f: AAGAGGAAACAGAAGCTCGCA r: TGCGAGCTTCTGTTTCCTCTT
exon 17	53217 (intron 17)	5.6 %	< 1 %	0.08 %	f: TGGGACCGTGCGCGTGTGGGG r: CCCCACACGCGCACGGTCCCA	**wt/mut:** f: TGGGACCGTGC/TGCGTGTGGGG r: CCCCACACGCG/ACACGGTCCCA **mut/mut:** f: TGGGACCGTGTGCGTGTGGGG r: CCCCACACGCACACGGTCCCA

ECE1 gene: Accession AL031005

exon 17	53220 (intron 17)	2.1 %	< 1 %	0.01 %	f: GACCGTGCGCGTGTGGGGAGC r: GCTCCCCACACGCGCACGGTC	**wt/mut:** f: GACCGTGCGCG/ATGTGGGGAGC r: GCTCCCCACAC/TGCGCACGGTC **mut/mut:** f: GACCGTGCGCATGTGGGGAGC r: GCTCCCCACATGCGCACGGTC
exon 19A	47813 (exon 19A)	2.1 %	< 1 %	0.01 %	f: GGCTCGGTTCCGGCTGAAAAC r: GTTTTCAGCCGGAACCGAGCC	**wt/mut:** f: GGCTCGGTTCC/TGGCTGAAAAC r: GTTTTCAGCCG/AGAACCGAGCC **mut/mut:** f: GGCTCGGTTCTGGCTGAAAAC r: GTTTTCAGCCAGAACCGAGCC

	Accession AL031728					
promA/ fragment 1	8625 (prom A1)	< 1 %	48.6 %	24%	f: GCCTGGTCCCGCTGCCCGGGT r: ACCCGGGCAGCGGGACCAGGC	**wt/mut:** f: GCCTGGTCCCG/TCTGCCCGGGT r: ACCCGGGCAGC/AGGGACCAGGC **mut/mut:** f: GCCTGGTCCCTCTGCCCGGGT r: ACCCGGGCAGAGGGACCAGGC
promA/ fragment 1	8938 (prom A1)	31.9 %	36.2 %	27%	f: TGCCCTCGATGTGGCCCAGAG r: CTCTGGGCCACATCGAGGGCA	**wt/mut:** f: TGCCCTCGATG/TTGGCCCAGAG r: CTCTGGGCCAC/AATCGAGGGCA **mut/mut:** f: TGCCCTCGATTTGGCCCAGAG r: CTCTGGGCCAAATCGAGGGCA
promA/ fragment 2	9351 (prom A2)	1.8 %	< 1 %	0.01 %	f: GCCCTCTCCTATAACCCTAGG r: CCTAGGGTTATAGGAGAGGGC	**wt/mut:** f: GCCCTCTCCTA/GTAACCCTAGG r: CCTAGGGTTAT/CAGGAGAGGGC **mut/mut:** f: GCCCTCTCCTGTAACCCTAGG r: CCTAGGGTTACAGGAGAGGGC
ECE1 gene: Accession AL031728						
promA/ fragment 2	9439 (prom A2)	25%	1.8 %	2%	f: CAAGAACCCCAGAGAGGTCTA r: TAGACCTCTCTGGGGTTCTTG	**wt/mut:** f: CAAGAACCCCA/CGAGAGGTCTA r: TAGACCTCTCT/GGGGGTTCTTG

						mut/mut: f: CAAGAACCCCCGAGAGGTCTA r: TAGACCTCTCGGGGGTTCTTG
	Accession AL031005					
						wt/mut:
promB/ fragment 1	109728 (promB1)	22.2 %	1.9 %	1.7 %	f: CAGTGACACCGTGTCATGTGC r: GCACATGACACGGTGTCACTG	f: CAGTGACACCG/ATGTCATGTGC r: GCACATGACAC/TGGTGTCACTG
						mut/mut: f: CAGTGACACCATGTCATGTGC r: GCACATGACATGGTGTCACTG
						wt/mut:
promB/ fragment 2	109249 (promB2)	20%	1.8 %	1.4 %	f: CATAATCCCACGCAAAAACAC r: GTGTTTTTGCGTGGGATTATG	f: CATAATCCCAC/TGCAAAAACAC r: GTGTTTTTGCG/ATGGGATTATG
						mut/mut: f: CATAATCCCATGCAAAAACAC r: GTGTTTTTGCATGGGATTATG

Table 2: This table includes all SNP's found in the 6 genes (EDN1, EDN-2, EDN-3, EDNRA, EDNRB and ECE-1), inclusive the genotype frequencies for heterozygotes and homozygotes for the mutant allele.

Table 3: new genomic sequences

gene	gene area (see figure 1 and 2)	Sequence positions in regard to reference sequences (see figure 1 and 2)	new sequences (5' to 3' orientation)
EDN2 gene:	**exon 1, 5'-UTR (intron 1)**	146-300	**146** CTGGC CTCACCTGCT GCCTACAGCT CCTGGGTCTG CCTATCTCTG TCTCCCTCTA GGGCTGCAGN GCCTCCCAAG CCAGCTGGGG GCAGACAGGG GCGGGAGCCT GGCGGCAGTG GTGGCAGGGG AAAGGTTCTG CAAATACAGA GGTGCCCAGG **300**
	exon 2 (intron 1)	1-209	**1** GCGGGAGCCT GGCGGCAGTG GTGGCAGGGG AAAGGTTCTG CAAATACAGA GGTGCCCAGG CTCCCCACGG GCTNGCTGGG ACTTCCCTGG GCTGGCCTGG AGCTCTTGAG CAGCAAGAGT GCCAACTCCC TCGATTATGC AAGAGGAGGG GCATGGTGGG TCCCCCACTC TGGGATGTGC CAGGCACCCC AACCCACCTC TCCACCTCT **209**
	exon 2 (intron 2)	403-540	**403** GATTGGGG GCAGGGGGAG CTCTGGGGCA GGGTGTCAGG GTGGAAGCTA CTGGCATGCT AGGGAACGTT GACACATTCC ATCTCTCCCT GGGCCTCCAG ATGGAGGCAT TGGCTAATAT CAATAGTTAA TTGTTTCAAT **540**
	exon 3 (intron	1-183	.....**1** AGAAGGGGAG GGTCTTGCCA AGGGCTACCA GGACCCTTGT

	2)		GGAGTAGCCT GTGACTTCAG TGGTGTGACT TCTTGAGGTC AAGGGCTGGT CACCTTTCAT TGGCACCCTG CTGGCATGGG TGGCTGTCCC AGTGGGCAGT GTGGACAGTC CCTGCTTGCC AATGGTTGTG GTTTATTTAA TCC **183**
	exon 3 (intron 3)	534-600	**534** GGCACCA TCCCTTCTTG CAGGAACCAG TCACCCATGG GGGCCTCCCG GTGCCCAGCT CTGGGGGCTT **600**
	exon 4 (intron 3)	1-160	.....**1** TCCTGGTAGC TCAGTTTTCC TCAGTCCCCA CCCCACCTGG AAAGGGAAAG ATGCCAGGTG CACAGGGGTG GAAATGGATC TGCTCCCGCA GGCAGGTGGG GATGGGGAAG GGGTGGGGGC TGGGCTGGGC CTGCAGCCAG GTAGCTTCAG GGCCCTGGGC **160**
	exon 4 (intron 4)	418-600	**418** GGA AAGCCAC**T**TG CCCAAAGTCA CACAGCAGTC AGCGGCATCT GGCTGACCCC AGAGTCTGTG CTCTTACCCA CTGTGCTCTT TGTTACCACT AGGCCTGGAA GTGGACTTTT TAGAGGGTTT GAAGGCTCAT GACTGGGAAG GACCACAGTT GTTCGTTCAG GCTGTGCTGA ATGGAGCATC **600**
EDN2 gene:	exon 5 (intron 4)	1-100	**1** TGTTTTCAGG CTGTGGTCAC TGTCTGACCC CAGGGCCTGC TGTGACCCCA GGATCTGGGC AAGGGCTGTA GCCCCATTGC CCCTGCTCTC ATGGGGAGCT **100**
EDNRB gene:	exon 4 (intron 3)	32-300	**32** TCCAAATAT TTCAAATTTT GCCTAACTAC TTAAGTTTTT GTGCATGCTT ATAACACATT GTCTTAGAGA ACTGAAATAT TTTTGGGAAA ATTTGTTTTA TTTAACATGA CTATTTATTT GTTCAGTAAG TGTGGCCTGA AAGATTCTGT ATGATATA**T**A CAAACTGGAT CTAATTTAGA AGATAATCAT TCCCTGATGA ATTTTTTTAA GTTTAACATT TGTTATATAA GATTTTCTTA CAGAGGAGTA TTAATCGTAA AAATTCTCTC **300**
	exon 4 (intron	471-700	**471** ACAAATATTT GATAACTCGT GGTTGAATTT ATAATTATGA

4)		ATATGAAAAT TATGATGATG ATGATGATAA ACTAACATTA TTATATACCA GAGCATATTT CCTTTTTCTT GTTTCTGGTA GTTTTAAATA GATAAAGAAT ATTTTTATAT TATTTATTTG GAGATATTCT ATATAATCTA AAAGGATCTA GGGAGAATCA GAACTTTTTA AAGATGAATC TCTGTCACTT **700**
exon 5 (intron 4)	3-240	**3** AATATTAT TTATTTGGAG ATATTCTATA TAATCTAAAA GGATCTAGGG AGAATCAGAA CTTTTTAAAG ATGAATCTCT AGTCACTTCG GTTCCACTTC ACATTAACTA TTCCATATAA AGCTCAGTGT CCTGAGTTTT TACATATGCC ACTGACTTTT TGTAGACAAT ATTAAATGTC GTTTTAGAAG ATAGAATGCT ATGAGTAAAA TGAGCCATCT TTTAAGGGTC AAACTATGGA **240**
exon 5 (intron 5)	395-668	**395** CAAACT AGAAATGGTT TTGTAAATAA ATGCCACTCA GAAGTGTTTC CATCGATCTT TTTTATAGGC AGAAAGACAG ACTACCATTT TTCTAACCCT TAGGAAGTCA TGGAGACTCC CAATTTTTAT CTGAGGTCCT GTTGAGAGGG ACAGGGAAGG GGGGGTATGA AGAACACTGG GGACAGAGAG TCAGAGTTGT CAAGATGATG AGTGAGTGCC AAGGCGGAAA ATGTCAGGTC CGATTCTCCA ACGCGTTTTC TAAATTTCTT CTTTTCAT **668**

Table 3: This table comprises sequences of the EDN2 gene and of the EDNRB gene, which represent new genomic sequence areas.

CLAIMS

1. A polynucleotide comprising a polynucleotide which is associated with at least one cardiovascular disease selected from the group consisting of :

(a) (i) a polynucleotide having the nucleic acid sequence of SEQ ID NO: 77, 78, 83, 84, 89, 90, 95, 96, 101 or 102;

(ii) a polynucleotide being capable of hybridizing to the EDN-1 gene, wherein said polynucleotide is having at a position corresponding to positions 3980 of the EDN-1 gene (Accession No: J05008) a C, at a position corresponding to position 5783 of the EDN-1 gene (Accession No: J05008) a G, at a position corresponding to position 9174 of the EDN-1 gene (Accession No: J05008) a TT, at a position corresponding to positions 10045 of the EDN-1 gene (Accession No: J05008) a C or at a position corresponding to position 10092 of the EDN-1 gene (Accession No: J05008) a C;

(b) (i) a polynucleotide having the nucleic acid sequence of SEQ ID NO: 107, 108, 113, 114, 119, 120, 125, 126, 131, 132, 137, 138, 143 or 144;

(ii) a polynucleotide being capable of hybridizing to the EDN-2 gene, wherein said polynucleotide is having at a position corresponding to position 57 of SEQ ID NO: 415 a T, at a position corresponding to position 32 of SEQ ID NO: 415 a G, at a position corresponding to position 181 of SEQ ID NO: 415 a A, at a position corresponding to position 133 of SEQ ID NO: 416 a A, at a position corresponding to position 190 of SEQ ID NO: 417 a C, at a position corresponding to position 428 of SEQ ID NO: 418 a C or at a position corresponding to position 287 of SEQ ID NO: 419 a A;

(c) (i) a polynucleotide having the nucleic acid sequence of SEQ ID NO:149, 150, 155, 156, 161 or 162;

(ii) a polynucleotide being capable of hybridizing to the EDN-3 gene, wherein said polynucleotide is having at a position corresponding to position 40215 of the EDN-3 gene (Accession No: AL035250) a

A, at a position corresponding to position 59430 of the EDN-3 gene (Accession No: AL035250) a C or at a position corresponding to position 63843 of the EDN-3 gene (Accession No: AL035250) a T;

(d) (i) a polynucleotide having the nucleic acid sequence of SEQ ID NO: 167, 168, 173, 174;

(ii) a polynucleotide being capable of hybridizing to the EDNRA gene, wherein said polynucleotide is having at a position corresponding to position 1366 of the EDNRA gene (Accession No: D11151) a deletion or at a position corresponding to position 1830 of the EDNRA gene (Accession No: D11151) a G;

(e) (i) a polynucleotide having the nucleic acid sequence of SEQ ID NO: 179, 180, 185, 186, 191, 192, 197, 198, 203, 204, 209, 210, 215, 216, 221, 222, 227 or 228;

(ii) a polynucleotide being capable of hybridizing to the EDNRB gene, wherein said polynucleotide is having at a position corresponding to position 749 of the EDNRB gene (Accession No: D13162) a G, at a position corresponding to position 937 of the EDNRB gene (Accession No: D13162) a C, at a position corresponding to position 1112 of the EDNRB gene (Accession No: D13162) a G, at a position corresponding to position 189 of SEQ ID NO: 420 a C, at a position corresponding to position 182 of SEQ ID NO: 421 a G, at a position corresponding to position 1048 of the EDNRB gene (Accession No: D13168) a A, at a position corresponding to position 1658 of the EDNRB gene (Accession No: D13168) a C, at a position corresponding to position 1912 of the EDNRB gene (Accession No: D13168) a T or at a position corresponding to position 2130 of the EDNRB gene (Accession No: D13168) a T;

(f) (i) a polynucleotide having the nucleic acid sequence of SEQ ID NO: 233, 234, 239, 240, 245, 246, 251, 252, 257, 258, 263, 264, 269, 270, 275, 276, 281, 282, 287, 288, 293, 294, 299, 300, 305, 306, 311, 312, 317, 318, 323, 324, 329, 330, 335, 336, 341, 342, 347, 348, 353, 354, 359, 360, 365, 366, 371, 372, 377, 378, 383, 384, 389, 390, 395, 396, 401, 402 or 422;

(ii) a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 423;

(iii) a polynucleotide being capable of hybridizing to the ECE-1 gene, wherein said polynucleotide is having at a position corresponding to position 86513 of the ECE-1 gene (Accession No: AL031005) a T, at a position corresponding to position 85627 of the ECE-1 gene (Accession No: AL031005) a A, at a position corresponding to position 85424 of the ECE-1 gene (Accession No: AL031005) a T, at a position corresponding to position 85472 of the ECE-1 gene (Accession No: AL031005) a G, at a position corresponding to position 83847 of the ECE-1 gene (Accession No: AL031005) a C, at a position corresponding to position 83924 of the ECE-1 gene (Accession No: AL031005) a T, at a position corresponding to position 75099 of the ECE-1 gene (Accession No: AL031005) a C, at a position corresponding to position 75102 of the ECE-1 gene (Accession No: AL031005) a T, at a position corresponding to position 75354 of the ECE-1 gene (Accession No: AL031005) a A, at a position corresponding to position 75431 of the ECE-1 gene (Accession No: AL031005) a deletion, at a position corresponding to position 73100 of the ECE-1 gene (Accession No: AL031005) a A, at a position corresponding to position 72974 of the ECE-1 gene (Accession No: AL031005) a A, at a position corresponding to position 65946 of the ECE-1 gene (Accession No: AL031005) a T, at a position corresponding to position 65875 of the ECE-1 gene (Accession No: AL031005) a T, at a position corresponding to position 61579 of the ECE-1 gene (Accession No: AL031005) a G, at a position corresponding to position 61752 of the ECE-1 gene (Accession No: AL031005) a C, at a position corresponding to position 56072 of the ECE-1 gene (Accession No: AL031005) a T, at a position corresponding to position 55860 of the ECE-1 gene (Accession No: AL031005) a A, at a position corresponding to position 55222 of the ECE-1 gene (Accession No: AL031005) a T, at a position corresponding to position 53127 of the ECE-1 gene

(Accession No: AL031005) a A, at a position corresponding to position 53217 of the ECE-1 gene (Accession No: AL031005) a T, at a position corresponding to position 53220 of the ECE-1 gene (Accession No: AL031005) a A, at a position corresponding to position 47813 of the ECE-1 gene (Accession No: AL031005) a T, at a position corresponding to position 8625 of the ECE-1 gene (Accession No: AL031728) a T, at a position corresponding to position 8938 of the ECE-1 gene (Accession No: AL031728) a T, at a position corresponding to position 9351 of the ECE-1 gene (Accession No: AL031728) a G, at a position corresponding to position 9439 of the ECE-1 gene (Accession No: AL031728) a C, at a position corresponding to position 109728 of the ECE-1 gene (Accession No: AL031005) a A, at a position corresponding to position 109249 of the ECE-1 gene (Accession No: AL031005) a T or at a position corresponding to position 1008 of the ECE-1 gene (Accession No: Z35307) a T:;

(iv) a polynucleotide encoding an ECE-1 polypeptide or fragment thereof, wherein said polypeptide comprises an amino acid substitution of Thr to Ile at position 324 of the sequence of the ECE-1 polypeptide (Accession No: CAA84548).

2. The polynucleotide of claim 1, wherein said cardiovascular disease is a coronary heart disease, hypertension, atherosclerosis or related to abnormal angiogenesis.

3. The polynucleotide of claim 1 or 2, wherein the encoded polypeptide is a member of the EDN/EDNR/ECE signaling system.

4. The polynucleotide of any one of claims 1 to 3 which is DNA or RNA.

5. A gene comprising the polynucleotide of any one of claims 1 to 3.

6. The gene of claim 5 which is further comprising any one of the

polynucleotides having the nucleic acid sequence of any one of SEQ ID NO: 403 to 410 or any one of SEQ ID NO: 411 to 414.

7. The gene of claim 5 or 6, wherein a nucleotide deletion, addition and/or substitution results in altered expression of the variant gene compared to the corresponding wild type gene.

8. A vector comprising a polynucleotide of any one of claims 1 to 4 or the gene of any one of claims 5 to 7.

9. The vector of claim 8, wherein the polynucleotide is operatively linked to expression control sequences allowing expression in prokaryotic or eukaryotic cells or isolated fractions thereof.

10. A host cell genetically engineered with the polynucleotide of any one of claims 1 to 4, the gene of any one of claims 5 to 7 or the vector of claim 8 or 9.

11. A method for producing a molecular variant polypeptide or fragment thereof which is associated with at least one cardiovascular disease comprising
(a) culturing the host cell of claim 10; and
(b) recovering said protein or fragment from the culture.

12. A method for producing cells capable of expressing a molecular variant polypeptide which is associated with at least one cardiovascular disease comprising genetically engineering cells with the polynucleotide of any one of claims 1 to 4, the gene of any one of claims 5 to 7 or the vector of claim 8 or 9.

13. A polypeptide or fragment thereof encoded by the polynucleotide of any one of claims 1 to 4, the gene of any one of claims 5 to 7 or obtainable by the method of claim 11 or from cells produced by the method of claim 12.

14. An antibody which binds specifically to the polypeptide of claim 13.

15. The antibody of claim 14 which specifically recognizes an epitope containing one or more amino acid substitution(s) resulting from a nucleotide exchange as defined in claim 1 or 7.

16. The antibody of claim 14 or 15 which is monoclonal or polyclonal.

17. A transgenic non-human animal comprising at least one polynucleotide of any one of claims 1 to 4, the gene of any one of claims 5 to 7 or the vector of claim 8 or 9.

18. The transgenic non-human animal of claim 17 which is a mouse, a rat or a zebrafish.

19. A solid support comprising one or a plurality of the polynucleotide of any one of claims 1 to 4, the gene of any one of claims 5 to 7, the vector of claim 8 or 9, the polypeptide of claim 13, the antibody of any one of claims 14 to 16 or the host cell of claim 10 in immobilized form.

20. The solid support of claim 19, wherein said solid support is a membrane, a glass- or poylpropylene- or silicon-chip, are oligonucleotide-conjugated beads or a bead array, which is assembled on an optical filter substrate.

21. An in vitro method for identifying a single nucleotide polymorphism said method comprising the steps of:

(a) isolating a polynucleotide of any one claims 1 to 4 or the gene of any one of claims 5 to 7 from a plurality of subgroups of individuals, wherein one subgroup has no prevalence for a cardiovascular disease and at least one or more further subgroup(s) do have prevalence for a cardiovascular disease; and

(b) identifying a single nucleotide polymorphism by comparing the nucleic acid sequence of said polynucleotide or said gene of said one

subgroup having no prevalence for a cardiovascular disease with said at least one or more further subgroup(s) having a prevalence cardiovascular disease.

22. A method for identifying and obtaining a pro-drug or a drug capable of modulating the activity of a molecular variant of a polypeptide of the EDN/EDNR/ECE signaling system comprising the steps of:

(a) contacting the polypeptide of claim 13, the solid support of claim 19 or 20, a cell expressing a molecular variant gene comprising a polynucleotide of any one of claims 1 to 4, the gene of any one of claims 5 to 7 or the vector of claim 8 or 9 in the presence of components capable of providing a detectable signal in response to drug activity with a compound to be screened for pro-drug or drug activity; and

(b) detecting the presence or absence of a signal or increase or decrease of a signal generated from the pro-drug or the drug activity, wherein the absence, presence, increase or decrease of the signal is indicative for a putative pro-drug or drug.

23. A method for identifying and obtaining an inhibitor of the activity of a molecular variant of a polypeptide of the EDN/EDNR/ECE signaling system comprising the steps of:

(a) contacting the protein of claim 13, the solid support of claim 19 or 20 or a cell expressing a molecular variant gene comprising a polynucleotide of any one of claims 1 to 4 or the gene of any one of claims 5 to 7 or the vector of claim 8 or 9 in the presence of components capable of providing a detectable signal in response to drug activity with a compound to be screened for inhibiting activity; and

(b) detecting the presence or absence of a signal or increase or decrease of a signal generated from the inhibiting activity, wherein the absence or decrease of the signal is indicative for a putative inhibitor.

24. The method of claim 22 or 23, wherein said cell is a cell of claim 10, obtained by the method of claim 12 or comprised in the transgenic non-human animal of claim 17 or 18.

25. A method of identifying and obtaining a pro-drug or drug capable of modulating the activity of a molecular variant of a polypeptide of the EDN/EDNR/ECE signaling system or its gene product comprising the steps of:

(a) contacting the host cell of claim 10, the cell obtained by the method of claim 12, the polypeptide of claim 13 or the solid support of claim 19 or 20 with the first molecule known to be bound by a protein of the EDN/EDNR/ECE signaling cascade to form a first complex of said polypeptide and said first molecule;

(b) contacting said first complex with a compound to be screened, and

(c) measuring whether said compound displaces said first molecule from said first complex.

26. A method of identifying and obtaining an inhibitor capable of the activity of a molecular variant of a polypeptide of the EDN/EDNR/ECE signaling system or its gene product comprising the steps of:

(a) contacting the host cell of claim 10, the cell obtained by the method of claim 12, the protein of claim 13 or the solid support of claim 19 or 20 with the first molecule known to be bound by a protein of the EDN/EDNR/ECE signaling cascade to form a first complex of said protein and said first molecule;

(b) contacting said first complex with a compound to be screened, and

(c) measuring whether said compound displaces said first molecule from said first complex.

27. The method of claim 25 or 26, wherein said measuring step comprises measuring the formation of a second complex of said protein and said compound.

28. The method of any one of claim 25 to 27, wherein said measuring step comprises measuring the amount of said first molecule that is not bound to said protein.

29. The method of any one of claims 25 to 28, wherein said first molecule is labeled.

30. A method for the production of a pharmaceutical composition comprising the steps of the method of any one of claims 22 to 29; and the further step of formulating the compound identified and obtained or a derivative thereof in a pharmaceutically acceptable form.

31. A method of diagnosing a disorder related to the presence of a molecular variant of a gene of the EDN/EDNR/ECE signaling system or susceptibility to such a disorder comprising determining the presence of a polynucleotide of any one of claims 1 to 4 or the gene of any one of claims 5 to 7 in a sample from a subject.

32. The method of claim 31 further comprising determining the presence of a polypeptide of claim 13 or the antibody of any one of claims 14 to 16.

33. A method of diagnosing a disorder related to the presence of a molecular variant of a gene of the EDN/EDNR/ECE signaling system or susceptibility to such a disorder comprising determining the presence of a polypeptide of claim 13 or the antibody of any one of claims 14 to 16.

34. The method of any one of claims 31 to 33, wherein said disorder is cancer, a cardiovascular disease or a disorder related to abnormal angiogenesis.

35. The method of any one of claims 31 to 34 comprising PCR, ligase chain reaction, restriction digestion, direct sequencing, nucleic acid amplification techniques, hybridization techniques or immunoassays.

36. A method of detection of the polynucleotide of any one of claims 1 to 4 or the gene of any one of claims 5 to 7 in a sample comprising the steps of
    (a) contacting the solid support of claim 19 or 20 with the sample under conditions allowing interaction of the polynucleotide of claim 1 to 4 or the gene of any one of claims 5 to 7 with the immobilized targets on a solid support and;
    (b) determining the binding of said polynucleotide or said gene to said immobilized targets on a solid support.

37. An in vitro method for diagnosing a disease comprising the steps of the method of claim 36, wherein binding of said polynucleotide or gene to said immobilized targets on said solid support is indicative for the presence or the absence of said disease or a prevalence for said disease.

38. A diagnostic composition comprising the polynucleotide of any one of claims 1 to 4, the gene of any one of any one of claims 5 to 7, the vector of claim 8 or 9, the polypeptide of claim 13 or the antibody of any one of claims 14 to 16.

39. A pharmaceutical composition comprising the polynucleotide of any one of claims 1 to 4, the gene of any one of claims 5 to 7, the vector of claim 8 or 9, the polypeptide of claim 13 or the antibody of any one of claims 14 to 16.

40. Use of the polynucleotide of any one of claims 1 to 4, the gene of any one of claims 5 to 7, the vector of claim 8 or 9, the polypeptide of claim 13, the polynucleotides having at a position corresponding to position 3745/6 of the EDN-1 gene (Accession No: J05008) an insertion of an A or at a position corresponding to position 7267 of the EDN-1 gene (Accession No: J05008) an A, or the antibody of any one of claims 14 to 16 for the preparation of a diagnostic composition for diagnosing a disease.

41. Use of the polynucleotide of any one of claims 1 to 4, the gene of any one of

claims 5 to 7, the vector of claim 8 or 9, the polypeptide of claim 13, or the antibody of any one of claims 14 to 16 for the preparation of a pharmaceutical composition for treating a disease.

42. The use of claim 40 or 41, wherein said disease is cancer or a cardiovascular disease comprising coronary heart disease, hypertension, atherosclerosis or a disease related to abnormal angiogenesis.

43. A diagnostic kit for detection of a single nucleotide polymorphism comprising the polynucleotide of any one of claims 1 to 4, the gene of any one of claims 5 to 7, the vector of claim 8 or 9, the polypeptide of claim 13, the antibody of any one of claims 14 to 16, the host cell of claim 10, the transgenic non-human animal of claim 17 or 18 or the solid support of claim 19 or 20.

Figure 1:

Reference sequence for EDN2:

Exon 1, 5'-UTR

- sequence, small letters and underlined: M65199 (exon 1, red letters, pos. 1-134) and sequence published in the literature (Sharma et al, 1998, Biochem. Biophys Res. Commun. 245: 709-712; black letters, pos. 135-145)
- sequence, capital letters: new and unpublished (pos. 146-300)
- primer sequences: small/capital blue letters in italic
-start codon: marked in green (box)

1 ccag***cttaat agcaggacgc tgg***caacagg cactccctgc tccagtccag cctgcgcgct

⇒

61 ccaccgccgc t[redacted]gtctcc gtgcctacca cctggtgctc cgttgcgcta gccctgctcg

121tggccctgca tgaaggtgag ctgccCTGGC CTCACCTGCT GCCTACAGCT CCTGGGTCTG

181CCTATCTCTG TCTCCCTCTA GGGCTGCAGN ***GCCTCCCAAG CCAGCTGG***GG GCAGACAGGG

⇐

241GCGGGAGCCT GGCGGCAGTG GTGGCAGGGG AAAGGTTCTG CAAATACAGA GGTGCCCAGG

Exon 2

- sequence, small letters and underlined: M65199 (exon 2, red letters, pos. 230-387) and sequence published in the literature (Sharma et al, 1998, Biochem. Biophys Res. Commun. 245: 709-712; black letters, pos. 210-229 and pos. 388-402)
- sequence, capital letters: new and unpublished (pos. 1-209 and pos.403-540)
- primer sequences: capital blue letters in italic

1 GCGGGAGCCT GGCGGCAGTG GTGGCAGGGG AAAGGTTCTG CAAATACAGA GGTGCCCAGG

61 CTCCCCACGG GCTNGCTGGG ACTTCCCTGG GCTGGCCTGG AGCT***CTTGAG CAGCAAGAGT***

⇒

121***GCC***AACTCCC TCGATTATGC AAGAGGAGGG GCATGGTGGG TCCCCCACTC
TGGGATGTGC

181CAGGCACCCC AACCCACCTC TCCACCTCTc tctccctcct gtgcatgcag
ggaagggcca

241ggctgctgcc accctggagc agccagcgtc ctcatctcat gcccaaggca
cccaccttcg

301gcttcgccgt tgctcctgca gctcctggct cgacaaggag tgcgtctact
tctgccactt

361ggacatcatc tgggtgaaca ctcctgagtg agcatccatg ggGATTGGGG
GCAGGGGGAG

421CTCTGGGGCA GGGTGTCAGG GTGGAAGCTA CTG***GCATGCT AGGGAACGTT***
GACACATTCC

⇐

481ATCTCTCCCT GGGCCTCCAG ATGGAGGCAT TGGCTAATAT CAATAGTTAA
TTGTTTCAAT

Figure 1 continued:

Exon 3

- sequence, small letters and underlined: M65199 (exon 3, red letters, pos. 261-384) and sequence published in the literature (Sharma et al, 1998, Biochem. Biophys Res. Commun. 245: 709-712; black letters, pos. 184-260 and pos. 385-533)
- sequence, capital letters: new and unpublished (pos. 1-183 and pos. 534-600)
- primer sequences: capital/small blue letters in italic

1 AGAAGGGGAG GGTCTTGCCA AGGGCTACCA GGACCCTTGT GGAGTAGCCT
GTGACTTCAG
61 TGGTGTGACT TCTTGAGGTC AAGGGCTGGT CACCTTTCAT T*GGCACCCTG*
*CTGGCATGG*G

⇒

121TGGCTGTCCC AGTGGGCAGT GTGGACAGTC CCTGCTTGCC AATGGTTGTG
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ccactacctt
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atagggtagc
421tgcaagcccg ggcatgccct ggggagcagg tgtctgggag actagcagag
gagcgaactg

⇐

481*gcaagttg*ggg ggtgagaagc gacccagggg cccacaggct gctttgggtc
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Exon 4

- sequence, small letters and underlined: M65199 (exon 4, red letters, pos. 196-294) and sequence published in the
literature (Sharma et al, 1998, Biochem. Biophys Res. Commun. 245: 709-712; blackletters, pos. 161-195 and pos. 295-417)
- sequence, capital letters: new and unpublished (pos. 1-160 and pos. 418-600)
- primer sequences: capital blue letters in italic

Figure 1 continued:

1 TCCTGGTAGC TCAGTTTTCC TCAGTCCCCA CCCCACCTGG AAAGGGAAAG ATGCCAGGTG

61 CACA***GGGGGTG GAAATGGATC TGC***TCCCGCA GGCAGGTGGG GATGGGGAAG GGGTGGGGGC

⇒

121TGGGCTGGGC CTGCAGCCAG GTAGCTTCAG GGCCCTGGGC agccatcact tgggccctaa

181tgctattcac gtaggactga agccggggca gtcccaagcc ggaagtcccc tgcagacgtg

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421AAGCCACTTG CCCAAAGTCA CACAGCAGTC AGCGGCATCT GGCTGACCCC AGAGTCTGT***G***

⇐

481***CTCTTACCCA CTGTGCTCTT*** TGTTACCACT AGGCCTGGAA GTGGACTTTT TAGAGGGTTT

541GAAGGCTCAT GACTGGGAAG GACCACAGTT GTTCGTTCAG GCTGTGCTGA ATGGAGCATC

Exon 5

- sequence, small letters and underlined: M65199 (exon 5, red letters, pos. 155-660) and sequence published in the literature (Sharma et al, 1998, Biochem. Biophys Res. Commun. 245: 709-712; black letters, pos. 101-154)
- sequence, capital letters: new and unpublished (pos. 1-100)
- primer sequences: capital/small blue letters in italic
- stop codon: marked in blue (box)

1 TGTTTTCAGG CTGTGGTCAC TGTCTGACCC CAGGGCCTGC TGTGACCCCA GGATCTGGGC

61 AAGGGCTGTA GCCC***CATTGC CCCTGCTCTC ATGG***GGAGCT cttggacagt ccatatctcc

⇒

121tgagatgcta agttgaccct tcttgattcc cagggacatt tccacagtca agagcctctt

181tgccaagcga caacaggagg ccatgcggga gcctcggtcc acacattcca ggtggaggaa

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541ctggaggctg cgt***cctgagg agtgtcctgt ctg***ctgggct acaaaccagg agcaaccgtg

⇐

601cagccacgaa cacgcatgcc tcagccagcc ctggagactg gatggctccc ctgaggctgg

Figure 2:

Reference sequence for EDNRB:

Exon 4

- sequence, small letters and underlined: D13165 (exon 4, red letters, pos. 311-460; intron 3, pos. 301-310 and intron 4, pos. 461-470, black letters each)
- sequence, capital letters: new and unpublished (pos. 32-300 and pos. 471-700)
- primer sequences: capital blue letters in italic

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  121 ATTTGTTTTA TTTAACATGA CTATTTATTT GTTCAGTAAG TGTGGCCTGA
AAGATTCTGT
                                                    ⇒
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  241 GTTTAACATT TGTTATATAA GATTTTCTTA CAGAGGAGTA TTAATCGTAA
AAATTCTCTC
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  541 ACTAACATTA TTATATACCA GAGCATATTT CCTTTTTCTT GTTTCTGGTA
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                              ⇐
  601 GATAAAGAAT ATTTTTATAT TATTTATTTG GAGATATTCT ATATAATCTA
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Exon 5

- sequence, small letters and underlined: D13166 (exon 5, red letters, pos. 251-384; intron 4, pos. 241-250 and intron 5, pos. 385-394, black letters each)
- sequence, capital letters: new and unpublished (pos. 3-240 and pos. 395-668)
- primer sequences: capital blue letters in italic

1 ..AATATTAT TTATTTGGAG ATATTCTATA TAATCTAAAA GGATCTAGGG AGAATCAGAA
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421 AT***GCCACTCA GAAGTGTTTC C***ATCGATCTT TTTTATAGGC AGAAAGACAG
ACTACCATTT
⇐
481 TTCTAACCCT TAGGAAGTCA TGGAGACTCC CAATTTTTAT CTGAGGTCCT
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541 ACAGGGAAGG GGGGGTATGA AGAACACTGG GGACAGAGAG TCAGAGTTGT
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<213> Homo sapiens

<400> 404
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ctccccacgg gctngctggg acttccctgg gctggcctgg agctcttgag cagcaagagt 120
gccaactccc tcgattatgc aagaggaggg gcatggtggg tcccccactc tgggatgtgc 180
caggcacccc aacccacctc tccacctct 209

<210> 405
<211> 138
<212> DNA
<213> Homo sapiens

<400> 405
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ggaacgttga cacattccat ctctccctgg gcctccagat ggaggcattg gctaatatca 120
atagttaatt gtttcaat 138

<210> 406
<211> 183
<212> DNA
<213> Homo sapiens

<400> 406
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tggtgtgact tcttgaggtc aagggctggt caccttttcat tggcaccctg ctggcatggg 120
tggctgtccc agtgggcagt gtggacagtc cctgcttgcc aatggttgtg gtttatttaa 180
tcc 183

<210> 407
<211> 67
<212> DNA
<213> Homo sapiens

<400> 407
ggcaccatcc cttcttgcag gaaccagtca cccatggggg cctcccggtg cccagctctg 60
ggggctt 67

<210> 408
<211> 160
<212> DNA
<213> Homo sapiens

<400> 408
tcctggtagc tcagttttcc tcagtcccca ccccacctgg aaagggaaag atgccaggtg 60
cacaggggtg gaaatggatc tgctcccgca ggcaggtggg gatggggaag gggtgggggc 120
tgggctgggc ctgcagccag gtagcttcag ggccctgggc 160

<210> 409
<211> 183
<212> DNA
<213> Homo sapiens

<400> 409
ggaaagccac ttgcccaaag tcacacagca gtcagcggca tctggctgac cccagagtct 60
gtgctcttac ccactgtgct ctttgttacc actaggcctg gaagtggact ttttagaggg 120
tttgaaggct catgactggg aaggaccaca gttgttcgtt caggctgtgc tgaatggagc 180
atc 183

<210> 410
<211> 100
<212> DNA
<213> Homo sapiens

<400> 410
tgttttcagg ctgtggtcac tgtctgaccc cagggcctgc tgtgacccca ggatctgggc 60
aagggctgta gccccattgc ccctgctctc atggggagct 100

<210> 411
<211> 269

<212> DNA
<213> Homo sapiens

<400> 411
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tcttagagaa ctgaaatatt tttgggaaaa tttgttttat ttaacatgac tatttatttg 120
ttcagtaagt gtggcctgaa agattctgta tgatatatac aaactggatc taatttagaa 180
gataatcatt ccctgatgaa ttttttttaag tttaacattt gttatataag attttcttac 240
agaggagtat taatcgtaaa aattctctc 269

<210> 412
<211> 230
<212> DNA
<213> Homo sapiens

<400> 412
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atgatgataa actaacatta ttatatacca gagcatattt ccttttttctt gtttctggta 120
gttttaaata gataaagaat atttttatat tatttatttg gagatattct atataatcta 180
aaaggatcta gggagaatca gaacttttta aagatgaatc tctgtcactt 230

<210> 413
<211> 238
<212> DNA
<213> Homo sapiens

<400> 413
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ttttaaagat gaatctctag tcacttcggt tccacttcac attaactatt ccatataaag 120
ctcagtgtcc tgagttttta catatgccac tgacttttttg tagacaatat taaatgtcgt 180
tttagaagat agaatgctat gagtaaaatg agccatcttt taagggtcaa actatgga 238

<210> 414
<211> 274
<212> DNA
<213> Homo sapiens

<400> 414
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ataggcagaa agacagacta ccatttttct aacccttagg aagtcatgga gactcccaat 120
ttttatctga ggtcctgttg agagggacag ggaaggggggg gtatgaagaa cactggggac 180
agagagtcag agttgtcaag atgatgagtg agtgccaagg cggaaaatgt caggtccgat 240
tctccaacgc gtttttctaaa tttcttcttt tcat 274

<210> 415
<211> 300
<212> DNA
<213> Homo sapiens

<400> 415
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tggccctgca tgaaggtgag ctgccctggc ctcacctgct gcctacagct cctgggtctg 180
cctatctctg tctccctcta gggctgcagn gcctcccaag ccagctgggg gcagacaggg 240
gcgggagcct ggcggcagtg gtggcagggg aaaggttctg caaatacaga ggtgcccagg 300

<210> 416
<211> 540
<212> DNA
<213> Homo sapiens

<400> 416
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ctccccacgg gctngctggg acttccctgg gctggcctgg agctcttgag cagcaagagt 120
gccaactccc tcgattatgc aagaggaggg gcatggtggg tcccccactc tgggatgtgc 180
caggcacccc aacccacctc tccacctctc tctccctcct gtgcatgcag ggaagggcca 240
ggctgctgcc accctggagc agccagcgtc ctcatctcat gcccaaggca cccaccttcg 300
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ctctggggca gggtgtcagg gtggaagcta ctggcatgct agggaacgtt gacacattcc 480
atctctccct gggcctccag atggaggcat tggctaatat caatagttaa ttgtttcaat 540

<210> 417
<211> 600
<212> DNA
<213> Homo sapiens

<400> 417
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tggctgtccc agtgggcagt gtggacagtc cctgcttgcc aatggttgtg gtttatttaa 180
tcctctctct ctttatttttt ttctctctct ctctttctct ccaccccaca ccactacctt 240
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cggcgccgct ccctgccaag gcgctgtcag tgctccagtg ccagggaccc cgcctgtgcc 360
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tgcaagcccg ggcatgccct ggggagcagg tgtctgggag actagcagag gagcgaactg 480
gcaagttggg ggtgagaagc gacccagggg cccacaggct gctttgggtc catggcacca 540
tcccttcttg caggaaccag tcacccatgg gggcctcccg gtgcccagct ctgggggctt 600

<210> 418
<211> 600
<212> DNA
<213> Homo sapiens

<400> 418
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cacaggggtg gaaatggatc tgctcccgca ggcaggtggg gatggggaag gggtgggggc 120
tgggctgggc ctgcagccag gtagcttcag ggccctgggc agccatcact tgggccctaa 180
tgctattcac gtaggactga agccggggca gtcccaagcc ggaagtcccc tgcagacgtg 240
ttccagactg gcaagacagg ggccactaca ggagagcttc tccaaaggct gaggtgaggg 300
cgtgggacac atgcacagac tgagcacctc ctgtatgcct tgcacatttc ccatattttc 360
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aagccacttg cccaaagtca cacagcagtc agcggcatct ggctgacccc agagtctgtg 480
ctcttaccca ctgtgctctt tgttaccact aggcctggaa gtggactttt tagagggttt 540
gaaggctcat gactgggaag gaccacagtt gttcgttcag gctgtgctga atggagcatc 600

<210> 419
<211> 660
<212> DNA
<213> Homo sapiens

<400> 419
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aagggctgta gccccattgc ccctgctctc atggggagct cttggacagt ccatatctcc 120
tgagatgcta agttgaccct tcttgattcc cagggacatt tccacagtca agagcctctt 180
tgccaagcga caacaggagg ccatgcggga gcctcggtcc acacattcca ggtggaggaa 240
gagatagtgt cgtgagctgg aggaacattg ggaaggaagc ccgcggggag agaggaggag 300
agaagtggcc agggcttgtg gactctctgc ctgcttcctg gaccggggcc ttggtcccag 360
acagctggac ccatttgcca ggattggcac aagctccctg gtgagggagc ctcgtccaag 420
gcagttctgt gtcctcgcac tgcccaggga agccctcggc ctccagactg cggagcagcc 480
tccagtgctg gctgctggcc cacagctctg ctggaagaac tgcatgggga gtacattcat 540
ctggaggctg cgtcctgagg agtgtcctgt ctgctgggct acaaaccagg agcaaccgtg 600
cagccacgaa cacgcatgcc tcagccagcc ctggagactg gatggctccc ctgaggctgg 660

<210> 420
<211> 669
<212> DNA
<213> Homo sapiens

<400> 420
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ttcagtaagt gtggcctgaa agattctgta tgatatatac aaactggatc taatttagaa 180
gataatcatt ccctgatgaa tttttttaag tttaacattt gttatataag attttcttac 240
agaggagtat taatcgtaaa aattctctca tccctatagt tttacaagac agcaaaagat 300
tggtggctgt tcagtttcta tttctgcttg ccattggcca tcactgcatt tttttataca 360
ctaatgacct gtgaaatgtt gagaaagaaa agtggcatgc agattgcttt aaatgatcac 420
ctaaagcagg taagaaaata caaatatttg ataactcgtg gttgaattta taattatgaa 480
tatgaaaatt atgatgatga tgatgataaa ctaacattat tatataccag agcatatttc 540
ctttttcttg tttctggtag ttttaaatag ataaagaata tttttatatt atttatttgg 600
agatattcta tataatctaa aaggatctag ggagaatcag aacttttaa agatgaatct 660
ctgtcactt 669

<210> 421
<211> 666
<212> DNA
<213> Homo sapiens

<400> 421
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ctcagtgtcc tgagttttta catatgccac tgacttttg tagacaatat taaatgtcgt 180
tttagaagat agaatgctat gagtaaaatg agccatcttt taagggtcaa actatggatt 240
tatttcagag acgggaagtg gccaaaaccg tcttttgcct ggtccttgtc tttgccctct 300
gctggcttcc ccttcacctc agcaggattc tgaagctcac tctttataat cagaatgatc 360
ccaatagatg tgaacttttg aggtaagaaa ggcaaactag aaatggtttt gtaaataaat 420
gccactcaga agtgtttcca tcgatctttt ttataggcag aaagacagac taccatttt 480
ctaaccctta ggaagtcatg gagactccca atttttatct gaggtcctgt tgagagggac 540
agggaagggg gggtatgaag aacactgggg acagagagtc agagttgtca agatgatgag 600
tgagtgccaa ggcggaaaat gtcaggtccg attctccaac gcgttttcta aatttcttct 660
tttcat 666

<210> 422
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic peptide

<220>
<221> CDS
<222> (1)..(18)

<400> 422
ctg cag atc ttg gca ccc 18
Leu Gln Ile Leu Ala Pro
1 5

<210> 423
<211> 6
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: synthetic peptide

<400> 423
Leu Gln Ile Leu Ala Pro
1 5

1

18